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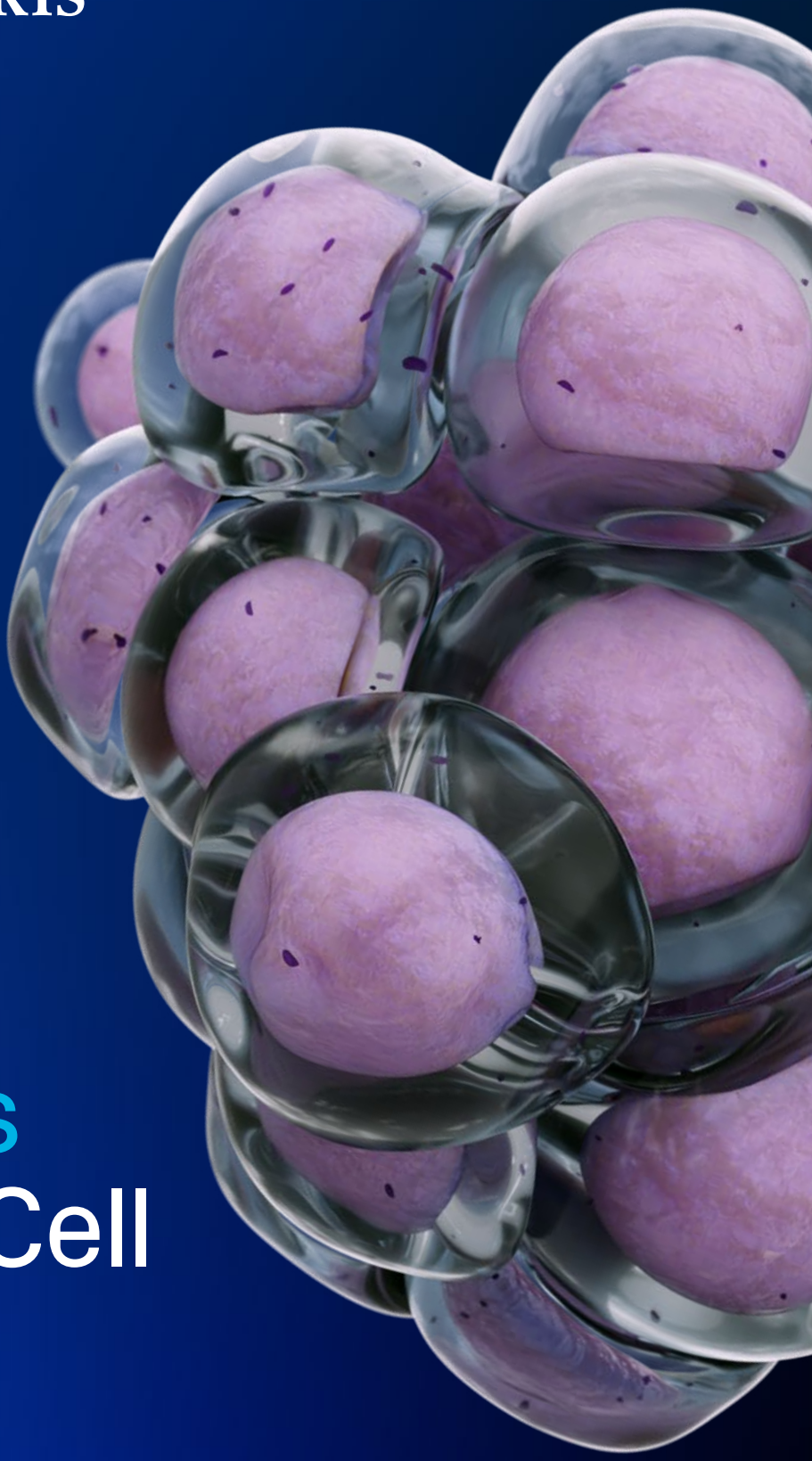
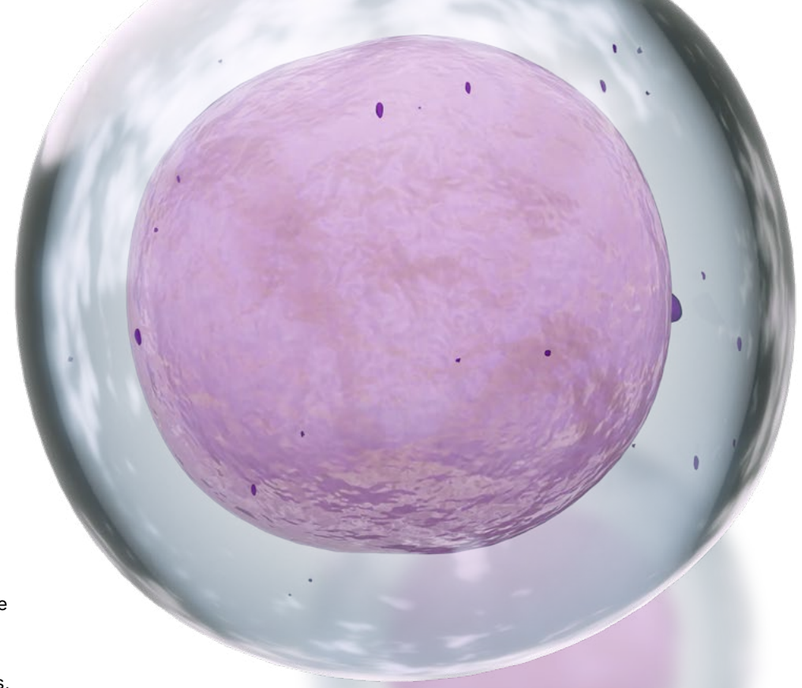
Edition 5



Small Molecules for Stem Cell Research

Stem Cell Research



Introduction

The defining characteristics of stem cells, i.e. their ability to self-renew and differentiate, make them a valuable resource in medical research and therapy. They are a potentially limitless source of cells for use in regenerative medicine. In addition, differentiating stem cells can provide insights into embryonic development and can generate organ-like structures, or organoids, which may help us to understand complex disease processes.

There are four main types of stem cells:

- **Embryonic stem cells** (ESCs) found in the inner cell mass of blastocysts, they give rise to all three primary germ layers of the developing embryo and are pluripotent, i.e. they can differentiate into any cell type;
- **Adult (or somatic) stem cells** found in adult tissues, they have more limited differentiation potential, and are important for tissue maintenance and repair;
- **Induced pluripotent stem cells** (iPSCs) produced by the reprogramming of somatic cells, e.g. skin fibroblasts;
- **Cancer stem cells** (CSCs), also known as tumor initiating cells, they are thought to be responsible for resistance and disease recurrence.

The discovery that somatic cells can be reprogrammed into iPSCs, with development potential indistinguishable from ESCs, has enabled the generation of disease-specific human iPSCs from patients with a disease of interest. This will help further our understanding of disease as well as permitting the development of patient-specific cell therapies, while bypassing the ethical issues associated with the use of ESCs.

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Why Small Molecules?

Stem cell proliferation and differentiation are controlled by signaling pathways and epigenetic mechanisms that can be readily manipulated. Cells can be reprogrammed or induced to differentiate into specific cell lines by introducing genes encoding transcription factors via retroviral transduction; Sendai viruses and plasmids have also been used for reprogramming. However, these techniques are time-consuming and carry the possibility of introducing genetic material or mutations into a cell's genome. Small molecules are increasingly being used alone or in combination with growth factors to modulate stem cells and are an essential component of the stem cell workflow.

The use of small molecules offers several advantages:

- **Easy to use:** Small molecules show effects within hours and greatly reduce the time associated with reprogramming and differentiation. In addition, good temporal control is possible as the effects of small molecules are rapid and reversible. Small molecules can also be used in combination with proteins such as growth factors and can improve the efficiency of reprogramming and differentiation techniques.
- **Synthetically produced:** Small molecules are chemically synthesized, and therefore have high purity and low batch-to-batch variation, ensuring consistent activity and reproducible results when used in stem cell culture. In contrast, proteins, which may also be used for cell reprogramming or differentiation, are manufactured via biological means. The chemically defined attributes of small molecules are an important safety consideration with respect to their use as ancillary reagents in cell therapy development.
- **Tunable:** The effects of small molecules in stem cells are concentration-dependent, so they can be used in different protocols with different outcomes.
- **Cell-Permeable:** Small molecules are cell-permeable, so can be used to target intracellular signaling pathways in both in vitro cell culture and in vivo.
- **Resembles natural process**: Using small molecules as "extrinsic" factors in the reprogramming or differentiation of stem cells resembles native processes more closely than the introduction of genes by viral transduction to induce these changes.

This brochure aims to provide a guide to the use of small molecules in stem cell research and the development of stem cell therapies. R&D Systems supplies Tocris™ small molecules for use at all stages of the stem cell workflow. Our small molecules are high quality and are widely cited in high-impact journals. With over 99.5% of products in stock, we offer rapid delivery and consistent resupply.

Signaling Pathways in Stem Cells

Multiple signaling pathways control the proliferation and differentiation of stem cells (SC). Small molecules that interact with these pathways are essential tools in stem cell biology, as they can be used to enhance and maintain the proliferation of stem cells, to direct the differentiation of stem cells towards more specialized cell types and to reprogram somatic cells to create populations of pluripotent stem cells.

Notch

The Notch signaling pathway is conserved across species from invertebrates to mammals and is important in the regulation of cell fate specification, proliferation and death in stem cells, and in the neuronal, cardiovascular and endocrine systems. It is important in the biological orientation of cells throughout development, allocating different cell types within a tissue. Activation of the pathway occurs when Delta or Jagged ligands expressed on the surface of neighboring cells induce cleavage of the Notch receptor by the membrane-associated protease γ-secretase. This results in release of the Notch intracellular domain (NICD), which translocates to the nucleus associating with the DNA binding protein CSL, leading to transcriptional activation. The outcome of Notch signaling is context-dependent, so that differences in the strength, timing, cell type, and context of the signal may affect the final outcome.

Notch is essential for maintaining neural progenitor cells in the developing brain. The potential for cells to differentiate to neurons, astrocytes and oligodendrocytes is maintained by the presence of Notch ligands. The γ-secretase inhibitor DAPT (Cat. No. 2634), prevents Notch pathway signaling allowing cells to commit to neuronal differentiation. Compound E (Cat. No. 6476), another γ-secretase inhibitor has also been used to induce neural differentiation and inhibit proliferation of ESCs.

Notch is important in hematopoiesis, directing hematopoietic stem cells into the T cell lineage and inhibiting the generation of B, NK and dendritic cells as well as monocytes. DAPT, which interferes with the Notch signaling pathway arrests T cell development and increases B cell numbers in hybrid human-mouse fetal thymus organ culture.

Canonical WNT

The Wnt family of secreted glycolipoproteins regulate diverse developmental processes such as differentiation, cell migration and proliferation during embryogenesis, and in adult tissues Wnt signaling is also important in tissue renewal. It is activated in ESCs and is downregulated during differentiation.

Signaling in the canonical Wnt pathway is stimulated by binding of Wnt protein to a receptor complex comprising a member of the Frizzled family of transmembrane receptors and a member of the LDL receptor family (LRP5/6). In the absence of Wnt binding to its receptor, the scaffolding proteins axin and adenomatous polyposis coli (APC) form a destruction complex with casein kinase 1α (CK1α) and glycogen synthase kinase-3β (GSK-3β). β-catenin, the main signaling molecule in the Wnt pathway, binds axin and is phosphorylated by CK1α and GSK-3β, targeting it for proteasomal degradation.

The effect of Wnt binding to the Frizzled receptor is relayed via the intracellular protein Dishevelled (Dvl/Dsh), to inhibit the destruction complex and release β-catenin. β-catenin then translocates to the nucleus where it complexes with T-cell factor/lymphoid enhancer factor (TCF/LEF) and promotes expression of transcription factors associated with pluripotency such as Oct 3/4. Wnt-induced β-catenin-mediated transcription drives stem cell self-renewal during adult tissue homeostasis and its dysregulation is associated with tumorigenesis and metastasis. Molecules that interact with this pathway could therefore be used to promote stem cell maintenance or drive differentiation.

BIO (Cat. No. 3194) is a small molecule that activates the Wnt signaling pathway by the potent and selective inhibition of GSK-3β, leading to sustained expression of pluripotent state-specific transcription factors Oct3/4, Nanog and Rex-1. It was the first pharmacological agent shown to maintain ESC self-renewal and its effects are similar to culturing cells in MEF-conditioned medium. The effects of BIO are reversible as removal of the compound allows stem cells to undergo normal differentiation. The GSK-3β inhibitor CHIR 99021 (Cat. No. 4423; also available as a trihydrochloride salt, Cat. No. 4953) used in combination with the MEK inhibitor PD 0325901 (Cat. No. 4192) maintain PSC self-renewal, while another potent and selective GSK-3β inhibitor, SB 216763 (Cat. No. 1616), has also been shown to maintain ESCs in a pluripotent state. Interestingly, both CHIR 99021 and the highly potent and selective GSK-3β inhibitor CHIR 98014 (Cat. No. 6695), also promote differentiation via activation of Wnt signaling.

Wnt proteins are secreted signaling proteins that undergo post-translational lipid modification prior to secretion. Porcupine (PORCN), a membrane-bound O-acyltransferase in the endoplasmic reticulum (ER), plays a key role in this post-translational processing of Wnt, so compounds that interfere with PORCN are likely to influence Wnt signaling. The PORCN inhibitors IWP 2 (Cat. No. 3533) and Wnt-C59 (Cat. No. 5148) both inhibit Wnt signaling and downregulate β-catenin target genes resulting in suppression of SC self-renewal and induction of differentiation.

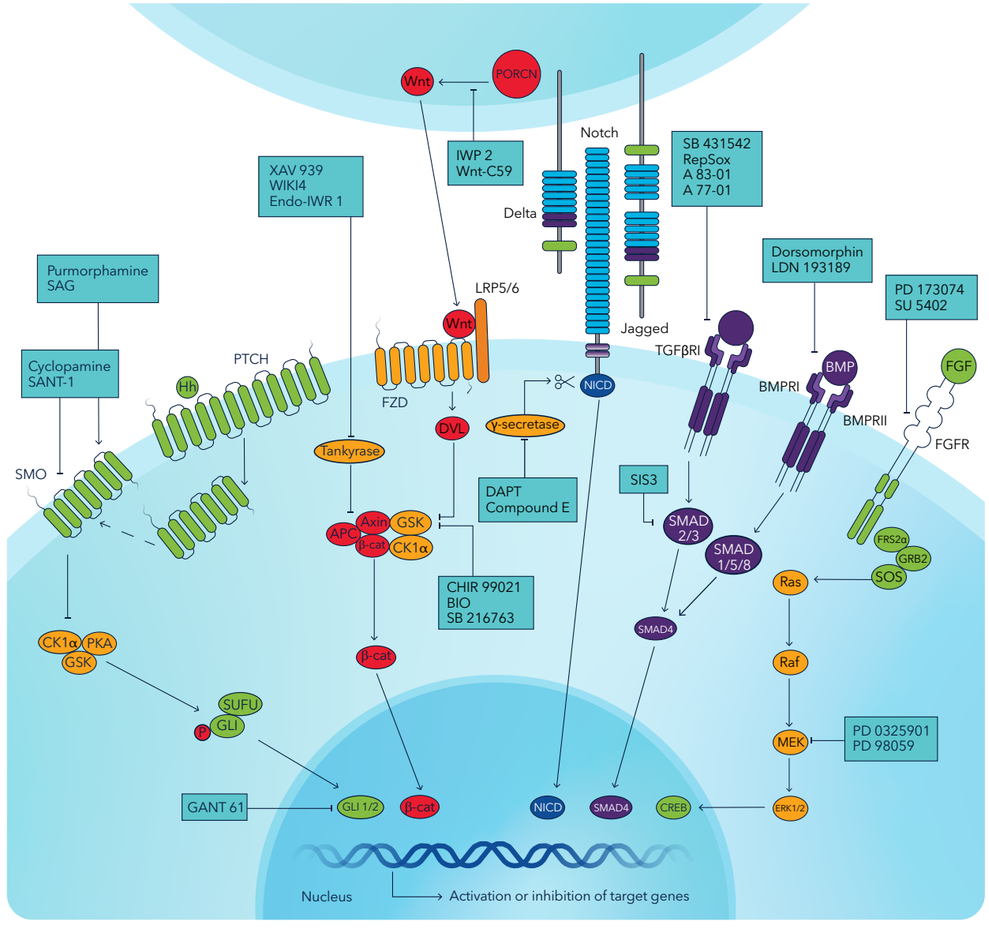


Figure 1. Key signaling pathways in stem cells. The proliferation and differentiation of stem cells are controlled by a network of signaling pathways. These pathways can be readily manipulated using small molecules (represented here in turquoise squares). Abbreviations: BMP, Bone morphogenetic protein; CK1α, casein kinase 1α; β-cat, β-catenin; DVL, Dishevelled; FGF, Fibroblast growth factor; FZD, Frizzled receptor; GSK, glycogen synthase kinase-3β; Hh, Hedgehog; NICD, Notch intracellular domain; PKA, protein kinase A; PORCN, Porcupine; PTCH, Patched receptor; SMO, Smoothened receptor; TGFβ, Transforming growth factor β.

Other compounds interact at different points in the Wnt signaling pathway to promote self-renewal of stem cells. Tankyrase is a poly ADP-ribosylating enzyme that interacts with axin, a component of the destruction complex, and stimulates its degradation through the ubiquitin-proteasome pathway, allowing β-catenin-mediated transcription. Inhibition of tankyrase by XAV 939 (Cat. No. 3748) stabilizes axin, leading to β-catenin degradation and inhibition of Wnt signaling. XAV 939 has been shown to promote cardiomyocyte differentiation from ESCs. Other tankyrase inhibitors, including WIKI4 (Cat. No. 4855) have also been shown to stabilize axin and block Wnt/β-catenin signaling, while *endo*-IWR 1 (Cat. No. 3532) has a similar effect.

In addition to its importance in stem cell maintenance, canonical Wnt signaling is also required for the proper cortex and hippocampus development. It induces the differentiation of neural progenitor cells during mid and late neurogenesis, as well as playing a key role in maintenance and repair in the adult brain.

How the same pathway can have these different roles in stem cell self-renewal/maintenance and differentiation, remains unclear, but it has been proposed that differential interactions between β-catenin and the transcriptional coactivators CREB-binding protein (CBP) or p300 activate transcriptional programs that promote either self-renewal or differentiation, respectively. How differential co-activator usage is controlled endogenously in adult stem/progenitor cell populations for normal tissue homeostasis and repair has not yet been determined but may involve coordination with the non-canonical Wnt signaling pathway.

Hedgehog

Hedgehog (Hh) proteins were first identified in *Drosophila* and were so called because Hh gene mutations resulted in the embryo surface resembling a hedgehog. The Hedgehog pathway is important in embryonic development in the patterning of many tissues and organs, including lung, bone, limbs and CNS. Hh signaling is also important in development of a range of cancers.

There are three hedgehog (Hh) proteins: Sonic (Shh), Indian (Ihh) and Desert (Dhh). Hh proteins bind the Patched (PTCH) transmembrane receptor. On ligand binding, PTCH is internalized and degraded, which releases the G protein-coupled receptor Smoothened (SMO) to the membrane where it inhibits phosphorylation of the suppressor of fused (SUFU)-glioma-associated oncogene homolog (GLI) complex by the kinases protein kinase A (PKA), CK1α and GSK-3β. This allows the dissociation of the SUFU-GLI complex, enabling translocation of the transcription factors GLI1 and GLI2 to the nucleus and activating transcription of Hh target genes, including PTCH1, MYC, IGF2, GLI1, GLI2. SAG (Cat. No. 4366) is a potent SMO agonist that activates the Hh pathway and enhances neuronal differentiation of hiPSCs into dopaminergic neurons. Purmorphamine (Cat. No. 4551), another SMO agonist, promotes osteogenesis of mesenchymal SCs.

In the absence of Hh, PTCH is inhibited, preventing SMO from translocating to the cell membrane. This allows the phosphorylation of the SUFU-GLI complex by PKA, CK1 and GSK-3β and subsequent inactivation by proteasomal degradation. Inactive GLI3-R then translocates to the nucleus where it inhibits transcription of Hh target genes.

Cyclopamine (Cat. No. 1623) inhibits the Hh pathway at the level of SMO by acting as an antagonist. This compound has been used in protocols to generate multipotent neural progenitors from mouse ESCs and to promote differentiation of pancreatic cells from human ESCs. Cyclopamine has also been found to deplete cancer stem cells in glioblastomas overexpressing GLI1. Another SMO antagonist, SANT-1 (Cat. No. 1974) has also been used to derive pancreatic β cells from PSCs. There are multiple sites in the Hedgehog signaling pathway that can act as targets for modulation by small molecules. GANT 61 (Cat. No. 3191) is a GLI antagonist that inhibits GLI dependent transcription of Hh target genes and inhibits pancreatic stem cell growth *in vitro*.

TGF-β/BMP

The transforming growth factor-beta (TGF-β) super family of proteins contains more than 30 secreted molecules including TGF-β, bone morphogenetic proteins (BMPs) and activins. The TGF-β/BMP signaling pathway is involved in many cellular processes and is a key regulator of pluripotency and cell-fate commitment from the earliest stages of embryonic development to homeostasis in adult tissues. BMP receptors are expressed on ESCs, and it has been shown that mESCs can be maintained in serum-free media supplemented with BMP-4 or BMP-2. The proper functioning of the TGF-β/BMP pathway depends on extensive crosstalk with other signaling pathways, including Wnt, Hh, Notch and mitogen-activated protein kinase (MAPK). Dysregulation of pathway activity leads to developmental defects and/or diseases, such as cancer.

TGF-β family members signal via transmembrane serine/threonine kinase receptors. There are 12 transmembrane kinase receptors subdivided into 7 type I and 5 type II receptors. Binding of TGF-β family ligands triggers the formation of a heterotetrameric complex consisting of two type I and two type II receptors. This induces transphosphorylation of type I by type II receptors, which in turn activates Smad signaling. There are eight mammalian Smads, which are transcriptional regulators. The receptor-regulated R-Smads, Smads 1, 2, 3, 5, and 8, are activated when they dock with phosphorylated type I receptors.
The TGF-β and activin receptors signal through Smad 2 and 3, whereas the BMP receptors signal through Smads 1, 5, and 8. The potent BMP4 agonist SB 4 (Cat. No. 6881), selectively activates BMP signaling and increases Smad 1/5/8 phosphorylation, with no effect on Smad2 or 3. IDE 1 (Cat. No. 4015) and IDE 2 are both reported to be activators of TGF-β signaling that induce Smad2 phosphorylation and have been used for definitive endoderm (DE) induction in human and mouse ESCs. Smad 6 and 7 provide an auto-inhibitory feedback mechanism. Interestingly, Smad1, 2 and 3 co-occupy the genome with the pluripotency factors Oct4, Nanog and Sox2.

Following binding of TGF-β to its receptor, the type I receptor activates Smad2 and 3, which form a complex with Smad4 and translocate to the nucleus where they regulate transcription of target genes through interaction with transcription factors and coactivators, such as the histone acetyltransferases CBP and p300.

A range of small molecules is available to interfere with TGF-β/BMP signaling at the receptor level. Many, such as SB 431542 (Cat. No. 1614) and RepSox (Cat. No. 3742), selectively inhibit type I receptors. Blockade of TGF-β type I receptors by SB 431542 promotes differentiation of hESCs, while both SB 431542 and RepSox can replace Sox2 in protocols to reprogram somatic cells to iPSCs. Dorsomorphin (Cat. No. 3093) and LDN 193189 (Cat. No. 6053) are both inhibitors of BMP type I receptors that promote differentiation of PSCs; Dorsomorphin, an inhibitor of ALK 2, ALK3, and ALK6, promotes cardiomyogenesis in mESCs, while LDN 193089, a potent and selective ALK2 and 3 inhibitor, has been shown to promote neural induction of PSCs when used in combination with SB 431542.

A 83-01 (Cat. No. 2939; and its active metabolite, A 77-01, Cat. No. 6712) inhibits TGF-βRI, ALK4 and ALK7 and has been shown to block Smad 2 phosphorylation and inhibit differentiation of rat iPSCs. It also increases clonal expansion efficiency and helps maintain homogeneity and long-term in vitro self-renewal of human iPSCs.

The TGF-β/BMP pathway may also be inhibited at the level of Smad. SIS3 (Cat. No. 5291) selectively inhibits Smad 3 preventing its interaction with Smad 4 and blocking TGF-β1-induced myofibroblast differentiation of fibroblasts.

FGF

The fibroblast growth factor (FGF) family of proteins is important in embryonic development and during organogenesis to maintain progenitor cells and control their growth, differentiation, survival, and patterning. FGFs also have a vital role in tissue repair and regeneration in adult tissues. Disruption of the FGF signaling pathway is associated with developmental defects, impaired response to injury, metabolic disorders and cancer.

FGFs and their receptors are highly conserved across species. The FGF family includes 23 proteins, 19 of which are secreted signaling proteins which bind to transmembrane tyrosine kinase receptors; the remaining four FGFs act intracellularly. Efficient receptor activation requires the binding of FGFs to heparan sulfate or other proteoglycans. There are four FGF receptors (FGFR1-4) which mediate signaling via four main intracellular pathways, the RAS-MAPK, the phosphatidylinositide 3-kinase/Akt pathway (PI3K-AKT) the phospholipase Cγ (PLCγ), and the STAT pathways, which are activated in a receptor- and cell type-dependent manner.

Binding of FGF ligands results in activation of the FGFR tyrosine kinase domain, leading to phosphorylation of the docked adaptor protein FGFR substrate 2α (FRS2α) and binding of other adaptor proteins, including PLCγ and STAT1, STAT3, and STAT5. Phosphorylated FRS2α recruits the adaptor protein GRB2, which in turn recruits the guanine nucleotide exchange factor SOS or the adaptor protein GAB1. SOS activates RAS-GTPase and the MAPK pathway, which results in activation of target transcription factors. GAB1 activates the PI3K-AKT pathway which has an inhibitory effect on target transcription factors.

Small molecule FGFR receptor inhibitors, such as PD 173074 (Cat. No. 3044) and SU 5402 (Cat. No. 3300), support mESC self-renewal. PD 173074 also enables the conversion of mouse epiblast stem cells to an earlier pluripotency state and inhibits differentiation of miPSCs to cardiomyocytes.

Compounds that interfere with the signaling pathways downstream of FGFR are also widely used in stem cell culture. PD 98059 (Cat. No. 1213), PD 0325901 (Cat. No. 4192), and U0126 (Cat. No. 1144) all inhibit the MAPK pathway at the level of MEK and have been shown to maintain stem cells in the undifferentiated state and to enhance the generation of iPSCs. Pluripotin is a dual ERK1/RasGAP inhibitor, that has been found to maintain ESC self-renewal.

Epigenetics in Stem Cells

Epigenetics can be defined as acquired changes in chromatin structure that arise independently of a change in the underlying DNA nucleotide sequence. Chromatin consists of a complex of DNA and histone proteins and one of its functions is to regulate gene expression and DNA replication. The basic unit of chromatin, the nucleosome, comprises two copies each of histone proteins H3, H4, H2A and H2B forming a core around which the DNA is wrapped. Another histone, H1 acts as a linker, binding the nucleosome at the entry and exit sites of the DNA. Epigenetic modifications, including DNA methylation, post-translation modification of histones, ATP-dependent chromatin remodeling and the activity of non-coding RNA, alter the accessibility of DNA to transcriptional machinery and therefore influence gene expression. Epigenetic modifications can be maintained and propagated through cellular division.

Regulation of the epigenome is coordinated via specific machinery: enzymatic complexes that catalyze specific DNA and chromatin modifications (writers); effector proteins that bind to these modifications (readers); and enzymes that remove the modifications (erasers). Epigenetic regulation to control expression of regulatory genes is key for the maintenance of the stem cell state. Disruption of the epigenetic machinery within stem cells may change the chromatin configuration and result in altered gene expression, leading to interruption of the self-renewal circuits and cell differentiation.

The epigenetic signature of ESCs is distinct from that of differentiated cells. ESCs have an open chromatin structure with characteristic DNA and histone modification profiles. This open structure enables transcription and is thought to be the key to pluripotency. During differentiation, cells undergo chromatin reorganization with the accumulation of more rigid heterochromatin resulting in highly condensed heterochromatin foci being prevalent in lineage-committed somatic cells. This results in the silencing of ESC-specific genes and other lineage specific genes. Consistent with this, repressive histone modifications are less prevalent in ESCs compared with differentiated cells, while active histone modifications are more abundant.

The reprogramming of somatic cells into iPSCs requires the complete reorganization of the epigenome, involving the resetting of the somatic epigenome into an ES cell-like state. The repressive epigenetic mechanisms that prevent unwanted gene expression in somatic cells represent ‘epigenetic barriers’ to somatic cell reprogramming. How these barriers function is poorly understood, but the use of small molecules to interfere with epigenetic modifications is helping to unravel the processes involved.

DNA Methylation

DNA methylation is an important epigenetic modification controlling gene transcription and has a key role in stem cell function. In stem cells, genes that control differentiation are methylated and transcriptionally inactive while genes essential for stem cell renewal are activated. DNA methylation commonly occurs at sites known as “CpG islands” - these are genomic areas rich in cytosine and guanine nucleotide base pairs that are frequently found near or at the promoter region of a gene. Methylation of cytosine residues within a transcription factor-binding element regulates gene transcription directly by preventing transcription factor binding, or via the action of methyl-CpG binding domain (MBD) containing proteins that preferentially bind methylated CpG dinucleotides and prevent gene expression. In order for differentiation to occur, DNA methylation at promoters of those genes involved in differentiation must be erased. Mutations in the machinery associated with DNA methylation in humans has been linked to certain neurological disorders, including Rett and Fragile-X syndromes, suggesting a role for DNA methylation in neural differentiation of stem cells. In somatic cells, DNA methylation is a repressive mechanism that helps prevent unwanted gene expression.

DNA methylation is controlled by DNA methyltransferase (DNMT) enzymes. DNMT inhibitors such as 5-Azacytidine (Cat. No. 3842) and RG 108 (Cat. No. 3295) induce demethylation and reactivation of silenced genes; they are used to enhance the efficiency of somatic cell reprogramming.

DNA demethylation can occur passively, but it is also carried out by cytidine deaminases and DNA glycosylases. Zebularine (Cat. No. 2293) is an inhibitor of cytidine deaminase that also acts as a DNMT inhibitor and has been found to potentiate the differentiation of mesenchymal stem cells to cardiomyocytes.

Sim *et al.* (2017) investigated the mechanism by which the 2i small molecule cocktail (see Self-renewal and Maintenance of Pluripotency section) maintains self-renewal and pluripotency of ESCs and found that inhibition of MEK and GSK-3β results in a global downregulation of DNA methylation. MEK inhibition by PD 0325901 (Cat. No. 4192) and GSK-3β inhibition by CHIR 99021 (Cat. No. 4423) both bring about a reduction in expression of DNMT3, but by different routes.

Histone Methylation

Histone methylation is another important PTM and this epigenetic modification is controlled by histone lysine methyltransferases (KMTs) and histone demethylases. Histone methylation can either repress or activate gene expression. For example, histone H3 trimethylated at lysine 27 (H3K27me3) represses expression of genes associated with development in PSCs, while methylation of H3 at lysine 4 (H3K4me3) activates expression of genes associated with pluripotency.

These conflicting histone modifications or marks may be co-localized at promoters of genes associated with differentiation in PSCs, suggesting that the genes are being silenced by H3K27me3, but these marks are erased in differentiating cells enabling the expression of lineage-specific genes.

Enhancer of zeste homolog 2 (EZH2) is a lysine methyltransferase and is the catalytic subunit of the polycomb repressive complex 2 (PRC2). It catalyzes the transfer of methyl groups from S-adenosylmethionine (SAM) to histone H3 at lysine 27. This facilitates the formation of heterochromatin and regulates gene expression and cell fate. In hESCs, EZH2 plays roles in the maintenance of pluripotency, self-renewal, proliferation and differentiation. 3-Deazaneplanocin A (DZNep; Cat. No. 4703) is an inhibitor of EZH2 that blocks trimethylation of lysine 27 on histone H3 and lysine 20 on histone H4 *in vitro*. The compound is widely used in chemical reprogramming protocols as it promotes expression of Oct4 in iPSCs. Induction of histone H3K9 hypomethylation using the G9a methyltransferase inhibitor BIX 01294 (Cat. No. 3364) also enhances the reprogramming of somatic cells into iPSCs. DOT1L is a KMT that methylates H3K79. The highly potent inhibitor EPZ 004777 (Cat. No. 5567) increases the efficiency of 4F-induced reprogramming of human fibroblasts by 3-4-fold, by increasing Nanog and Lin28 levels. EPZ 004777 can also be used in 2-factor reprogramming using Oct4 and Sox2.

Reprogramming efficiency can also be enhanced by Tranylcypromine (Cat. No. 3852), an inhibitor of lysine-specific histone demethylase 1A (LSD1).

Bromodomains

Bromodomains (BRDs) are epigenetic “readers” that selectively recognize acetylated lysine residues on histone protein tails. The BET (bromodomain and extra-terminal) bromodomain family, which comprises the ubiquitously expressed proteins BRD2, BRD3, BRD4, and the testis-specific protein, BRDT, play a key role at the interface between chromatin remodeling and transcriptional regulation. BET bromodomains are integral in the regulation of transcriptional memory, the mechanism by which cells “remember” their unique transcriptional program following division. Genes regulated by BET are important in controlling cell identity; BRD4 for example is important in the control of transcriptional

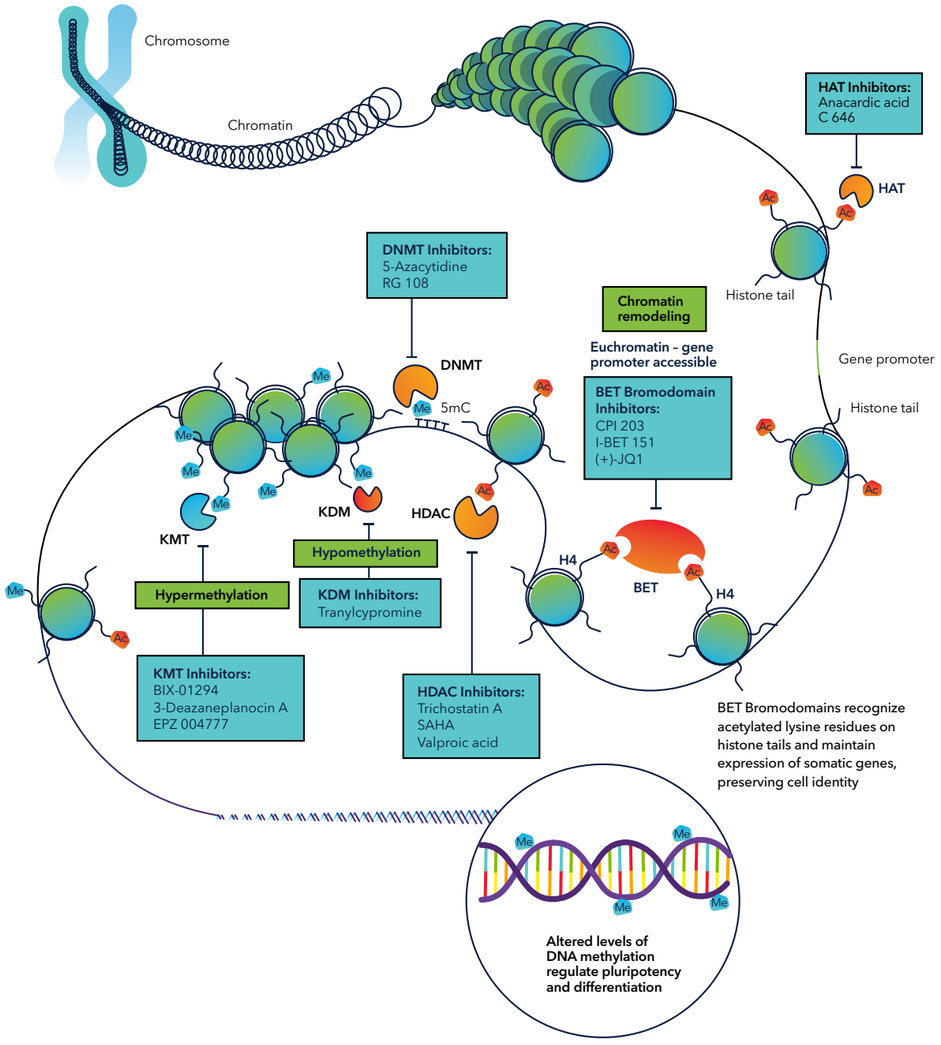


Figure 2. Key epigenetic mechanisms regulating stem cells. The fundamental unit of chromatin is the nucleosome, which consists of an octamer of the histone proteins H2A, H2B, H3 and H4 (two of each) tightly bound by DNA. Alterations in chromatin structure by post-translational modifications can regulate gene expression through the formation of heterochromatin or euchromatin, which usually repress or activate gene transcription, respectively. Post-translational modifications include DNA methylation and methylation (Me) and acetylation (Ac) of histone tails. DNA methylation and the methylation or acetylation of histone tails can repress or promote gene expression, depending on the site and extent of methylation/acetylation, as well as the presence of other histone modifications in the vicinity. The pattern of these post-translational modifications on a nucleosome determines the transcriptional profile of nearby genes. Abbreviations: DNMT, DNA methyltransferases; HAT, histone acetyltransferases; HDAC, histone deacetylases; KDM, histone demethylases; KMT, lysine methyltransferases.

elongation of pluripotency genes and maintaining the identity of ESCs. I-BET 151 (Cat. No. 4650) blocks recruitment of BRD3/4 to chromatin, and has been found to promote differentiation of hiPSCs into megakaryocytes. However, when used at a low concentration, I-BET 151 promotes reprogramming. Li *et al.* (2015) found that I-BET 151 was required in their protocol to transdifferentiate murine fibroblasts to neurons, to disrupt the fibroblast core transcriptional network and suppress endogenous fibroblast fate-determining programs (see Transdifferentiation section). Similarly, the BET bromodomain inhibitors, (+)-JQ1 (Cat. No. 4499) and CPI 203 (Cat. No. 5331) also enhance 4F reprogramming when used at lower concentrations (Shao *et al.* 2016). In addition, inhibition of BET bromodomains downregulates somatic genes in naive fibroblasts as well as during reprogramming and results in loss of fibroblast morphology.

Reprogramming

The regression of a specialized cell to a simpler state, resulting in cells with stem-like properties, is known as dedifferentiation and is a process that occurs naturally, mostly for repair and regeneration in aged or damaged tissues. The generation of cells with stem-like properties from specialized cells can also be induced in the laboratory, where it is known as reprogramming.

In 2006, Takahashi and Yamanaka published the results of research investigating whether certain transcription factors known to function in the maintenance of pluripotency in ESCs could be used to induce pluripotency in somatic cells. Their findings were the first to show the successful reprogramming of mouse embryonic and adult fibroblasts using defined factors, into cells with stem-like properties, known as induced pluripotent stem cells (iPSCs). The researchers (Takahashi *et al.* 2007) then took adult human dermal fibroblasts and introduced retroviruses containing the transcription factors Oct3/4, Sox2, Klf4 and c-Myc (known as the Yamanaka factors or OSKM), and grew the cells in embryonic stem cell (ESC) culture medium. The resulting human iPSCs were found to have similar properties to hESCs and could differentiate into all three embryonic germ layers, presenting the prospect of generating autologous stem cells for cell therapy applications for the first time.

The introduction of retroviruses into cells to deliver the Yamanaka factors is associated with concerns over genome modification, and the intervening years have seen the development of other methods to deliver reprogramming factors, such as the use of plasmids and reprogramming mRNAs. Such techniques are still widely used, but Yamanaka factor reprogramming is slow and has very low efficiency (approximately 0.01 – 0.02% conversion rate over 30 days. Huangfu *et al.* (2008) screened small molecules for their ability to improve the efficiency of reprogramming and found that the histone deacetylase (HDAC) inhibitor Valproic acid (VPA, Cat. No. 2815), can increase OSKM reprogramming efficiency by >100-fold.

Subsequent research has sought to replace exogenous expression of the Yamanaka factors with small molecules, since they have several advantages including being cell permeable, non-immunogenic and readily synthesized. Several compounds have been identified, using both phenotypic screening and hypothesis-driven research, which can functionally replace Yamanaka factors in reprogramming. Huangfu *et al.* found that VPA allows the efficient induction of iPSCs without the oncogene c-Myc. Other groups discovered additional small molecules that substitute for transcription factors, including Lyssiotis *et al.* (2009), who showed that MEFs could be reprogrammed using a GSK-3β inhibitor, such as Kenpaullone (Cat. No. 1398), in place of Klf4 and Ichida *et al.* (2009), who discovered that inhibition of TGF-β signaling by SB 431542 (Cat. No. 1614) or RepSox (Cat. No. 3742) can replace Sox-2.

Hou *et al.* (2013) first described the generation of iPSCs without transcription factors, using only a cocktail of small molecules. The researchers discovered that a combination of six compounds, VPA, CHIR 99021 (Cat. No. 4423), RepSox, Tranylcypromine (Cat. No. 3852), Forskolin (Cat. No. 1099) (these five compounds are together known as VC6TF) and 3-Deazaneplanocin A (Cat. No. 4703) can

be used to reprogram mouse embryonic fibroblast (MEFs) cells in around 28 – 36 days at a frequency of 0.2%, dispensing with the need for transfection with exogenous master genes. The resulting chemically-induced PSCs, or ciPSCs, resembled ESCs with respect to gene expression profiles, epigenetic status and differentiation potential.

Zhao *et al.* (2015, 2018) further modified this protocol to create a more efficient method for the generation of ciPSCs from MEFs. Their three-stage process used a cocktail of 12 small molecules plus the growth factors, leukemia inhibitory factor (LIF; R&D Systems: 7734-LF) and basic fibroblast growth factor (bFGF; R&D Systems: 233-FB) to generate ciPSCs in around 16 to 20 days (FIGURE 3). In the first stage, MEFs were cultured in an optimized medium containing VC6TF plus the retinoic acid receptor agonist Ch 55 (Cat. No. 2020) and lysine methyltransferase (KMT) inhibitor EPZ 004777 (Cat. No. 5567) for 4-6 days. This resulted in the formation of small extraembryonic endoderm (XEN)-like colonies. The intermediate XEN-like cells resembled embryo-derived XEN cells in their gene expression profiles, their reprogramming potential and in vivo development potential. In stage 2, the XEN-like cells were cultured for a further 4-6 days with the addition of Decitabine (Cat. No. 2624) plus SGC 0946 (Cat. No. 4541), another KMT inhibitor, replacing EPZ 004777. The culture medium was also supplemented with LIF, bFGF, and L-Ascorbic acid (Cat. No. 4055). The use of SGC 0946 in place of EPZ 04777 at this stage increased reprogramming efficiency by 5-fold. In the third stage, the cells were grown in medium containing CHIR 99021 and PD 0325901 (Cat. No. 4192) (2i condition – see Self-Renewal chapter). The MEK inhibitor PD 0325901 is key in stage 3 to activate the expression of the pluripotency associated genes Nanog and Sox2. Their modified protocol increased the yield of ciPSC colonies by 1000-fold compared with the earlier version (Hou *et al*. 2013) and reduced the duration of the process to as little as 20 days.

Chemical repogramming techniques have subsequently been adapted for use in human cells. A paper by Liuyang *et al*. (2023) describes a 3-stage protocol for the generation of ciPSCs from human adult adipose-derived stromal cells (hADSCs) in serum-free, chemically-defined conditions.

The JNK pathway has been identified to be a major barrier to chemical reprogramming, and inhibition of the JNK pathway is essential for induction of somatic cell dedifferentiation. In stage I, a combination of small molecules promoted the emergence of epithelial-like cells expressing LIN28A, a gene important in regulating dedifferentiation and regeneration, in around 8 days; in stage II the cells entered an intermediate plastic state; and in stage III, fully reprogrammed ciPSCs were generated. The researchers demonstrated the protocol to be rapid (approximately 30 days), reproducible and with a reprogramming efficiency of up to 31% (FIGURE 4).

There has been some uncertainty over whether iPSCs are completely equivalent to ESCs in function and character. The conversion of somatic cells to iPSCs involves reprogramming of the epigenome and since iPSCs have similar properties to ESCs, it should be expected that the epigenome of the iPSCs resembles that of ESCs. Ping *et al*. (2018) compared the DNA methylation states of mouse ciPSCs, OSKM-iPSCs and ESCs and found that ciPSCs are more hypomethylated than OSKM-iPSCs and have a DNA methylation pattern closer to mESCs. This is important, because aberrant DNA methylation states of iPSCs can be transmitted through differentiation, which has implications for the use of iPSCs in both cell therapy and research.

The reprogramming of MEFs to ciPSCs through the intermediate stage of XEN-like cells is not seen with OSKM reprogramming, but Zhao *et al*. found that it is essential for the generation of ciPSCs. Their modified protocol increased the yield of ciPSC colonies by 1000-fold compared with the earlier version (Hou *et al.* 2013) and reduced the duration of the process to as little as 20 days.

Lister *et al*. (2011) compared the DNA methylation patterns of Yamanaka factor derived (4F) hiPSCs, hESCs and somatic cells (foreskin fibroblasts) and found that on a genome scale the DNA methylomes for ESCs and hiPSCs are similar and differ from that of somatic cells. iPSCs and ESCs show higher frequency methylation at both CpG and non-CpG dinucleotides than somatic cells. However, in depth analysis of DNA methylation revealed numerous differently methylated regions (DMRs) between ES and iPS cell lines. These differences may be the result of a failure to fully reprogram the somatic cell methylation patterns, or they may be iPSC-specific DMRs. In addition, these aberrant DNA methylation states of iPSCs are transmitted through differentiation, which has implications for the use of iPSCs.

Ping *et al*. (2018) compared the DNA methylation status of mouse ciPSCs, 4F-iPSCs and ESCs and found that ciPSCs are more hypomethylated than 4F-iPSCs and have a DNA methylation pattern closer to mESCs, suggesting that chemical reprogramming might be better than transcription factor reprogramming.

This type of chemical reprogramming therefore holds great promise for the generation of autologous or allogeneic stem cells for regenerative medicine, as well as for disease modeling.

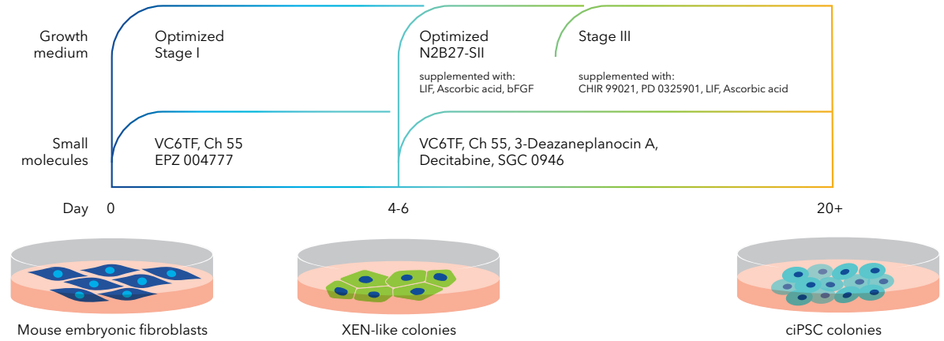
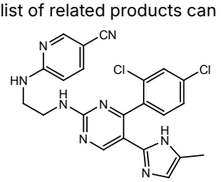
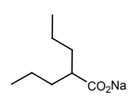


Figure 3. Schematic outlining a protocol for the highly efficient generation of ciPSCs from MEFs using a cocktail of 12 small molecules. From Zhao *et al.* (2018) Cell Stem Cell **23**:31.

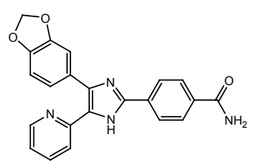
BOX 1: Reprogramming Products
A full list of related products can be found on pages 28-36



CHIR 99021 (4423)
Enables reprogramming of MEFs to iPSCs; enhances ESC self-renewal; also commonly used in organoid generation; GSK-3β inhibitor. GMP version available



Valproic Acid (2815)
Enables induction of pluripotent stem cells from somatic cells; histone deacetylase inhibitor



SB 431542 (1614)
Replaces Sox2 in reprogramming of fibroblasts into iPSCs; potent and selective TGF-βRI inhibitor. GMP version available

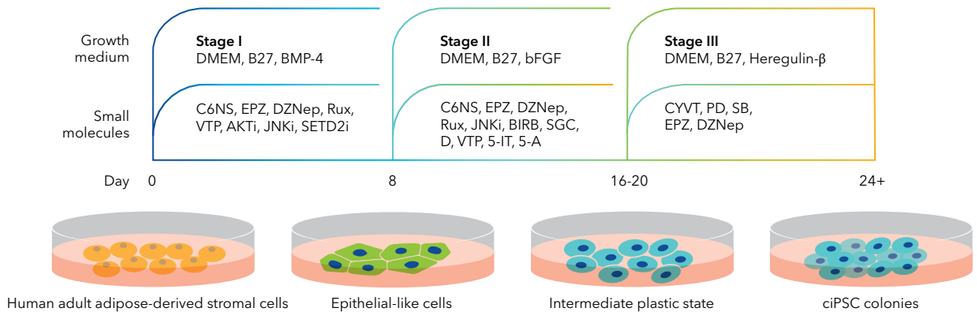


Figure 4. Schematic outlining a protocol for the highly efficient generation of human ciPSCs from adult adipose-derived stromal cells in chemically defined conditions. From Liuyang *et al.* (2023) Cell Stem Cell 30:1. C, CHIR 99021 (Cat. No. 4423); 6, RepSox (Cat. No. 3742); N, TTNPB (Cat. No. 0761); S, SAG (Cat. No. 4366); EPZ, EPZ 5676; DZNep, 3-Deazaneplanocin A (Cat. No. 4703); Rux, Ruxolitinib (Cat. No. 7064); VTP, VTP 50469; AKTi, AKT inhibitor; JNKi, JNKIN8; SETD2i, SETD2-IN-1; BIRB, BIRB 796 (Cat. No. 5989); SGC, SGC-CBP30 (Cat. No. 4889); D, Dorsomorphin (Cat. No. 3093); 5-IT, 5-Iodotubericin (Cat. No. 1745); 5-A, 5-Azacytidine (Cat. No. 3842); Y, Y-27632 (Cat. No. 1254); V, Valproic acid (Cat. No. 2815); T, Tranylcypromine (Cat. No. 3852); PD, PD 0325901 (Cat. No. 4192); SB, SB 590885 (Cat. No. 2650).

Self-Renewal and Maintenance of Pluripotency

Embryonic stem cells are pluripotent cells that self-renew and proliferate via a process involving the division of a parent cell into two identical daughter cells, while also having the capacity to generate all cell lineages of the developing and adult organism. The isolation and culture of mouse embryonic stem cells was first described in 1981 by two separate labs. Evans and Kaufman isolated mESCs from the inner cell mass of mouse blastocysts and cultured them in vitro on a fibroblast feeder layer, while Martin also isolated cells from mouse blastocysts, but cultured them in medium conditioned by a teratocarcinoma cell line. The researchers postulated that the conditioned medium contained factors that stimulated the proliferation or inhibited the differentiation of the ESCs. This was subsequently found to be the case. The factors were identified as LIF (leukemia inhibitor factor), Wnt and TGF-β/BMP (transforming growth factor-β/bone morphogenetic protein) signaling pathway ligands for mouse ESCs. In humans, FGF2 (fibroblast growth factor 2) and Activin signaling pathways are also important.

The maintenance of stem cells in the undifferentiated pluripotent state is controlled by a range of intrinsic and extrinsic factors including signaling pathways and growth factors. Extensive research has established a definition of ESCs in terms of gene regulation and revealed that the stable expression of three core transcription factors, Oct4, Sox2 and Nanog, are key to maintaining pluripotency and self-renewal.

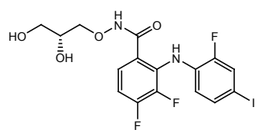
Oct4, Sox2 and Nanog occupy and enhance the activity of genes associated with maintaining the pluripotent state, while repressing genes that enable differentiation. These core transcription factors function together and form an autoregulatory loop. When all three factors are expressed at the appropriate levels, the autoregulatory loop functions as a positive feedback control of gene expression that maintains ESCs in the pluripotent state. However, if the expression of one of the transcription factors is altered, gene expression is switched to a differentiation program. DNA sites occupied by Oct4, Sox2 and Nanog are also co-occupied by Stat3, Tcf3 and Smad1, which are the target transcription factors of the LIF, Wnt and TGF-β/BMP pathways, respectively. This allows for control of the core factors and therefore self-renewal/differentiation by these signaling pathways.

Conventional stem cell culture techniques require mouse embryonic fibroblast (MEF) 'feeder' cells, serum products and growth factors, such as LIF and bFGF. These methods have several disadvantages including that certain components are not fully defined or are animal-derived (e.g. fetal bovine serum or FBS) products, which have the potential to introduce unwanted animal pathogens into stem cell culture. Recently, chemically-defined serum-free media have been developed to replace the requirement for feeder cells and serum products. It has been shown that stem cells can be maintained in culture without feeder cells, by supplementation with LIF and/or BMP. LIF alone can maintain mESC self-renewal, via activation of the LIF-Stat3 pathway, however, Sato *et al.* (2004) showed that it is not enough to prevent differentiation of hESCs, suggesting that other pathways are also required to be activated. Sato *et al.* investigated whether the Wnt signaling pathway has a role in maintaining SC pluripotency. The researchers found that activation of the Wnt signaling pathway by inhibition of GSK-3β using the small molecule BIO (Cat. No. 3194), maintained hESCs and mESCs in the undifferentiated state and sustained the expression of the pluripotency associated transcription factors Oct-3/4, Rex-1 and Nanog. BIO has also been found to be useful in promoting self-renewal of cardiovascular progenitors.

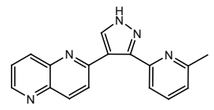
In 2006, Chen *et al.* identified a small molecule, SC 1 (also known as Pluripotin), which can be used to maintain pluripotency of mESCs in the absence of feeder cells, serum and LIF. SC 1 inhibits differentiation mechanisms, via inhibition of ERK1 and RasGAP, rather than promoting self-renewal.

Ying *et al.* (2008) postulated that the LIF and BMP signals act downstream from ERK to block ESC commitment. To test this theory, they cultured mESCs in a combination of LIF with the small molecule MEK inhibitor PD 184352 (Cat. No. 4237) and the FGF receptor tyrosine kinase inhibitor SU 5402 (Cat. No. 3300), as a substitute for serum/BMP and found that the combination could support ESC proliferation. However, occasional neural rosettes appeared, and apoptosis was relatively high using this combination. As it had previously been demonstrated that a GSK-3β inhibitor (BIO) could maintain self-renewal, Ying *et al.* then explored whether adding the more selective GSK-3β inhibitor CHIR 99021 (Cat. No. 4423) could enhance growth of ESCs cultured in a combination of PD 184352 and SU 5402. It was found that the combination of these three inhibitors (3i) led to expansion of ESC colonies for several weeks with a doubling rate comparable to that in LIF/serum/BMP.

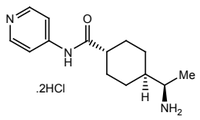
BOX 2: Self-renewal Products
A full list of related products can be found on pages 28-36.



PD 0325901 (4192)
Maintains self-renewal of ESCs in combination with CHIR 9902; potent MEK1/2 inhibitor



RepSox (3742)
Enhances reprogramming efficiency; replaces Sox2; Potent and selective inhibitor of the TGF-βRI



Y-27632 (1254)
Reduces apoptosis of PSCs during routine passage and improves survival of stem cells undergoing cryopreservation; ROCK inhibitor. GMP version available

Replacing PD 184352 and SU 5402 with the more potent MEK inhibitor PD 0325901 (Cat. No. 4192), to achieve more effective inhibition of ERK activation, it was subsequently shown to be sufficient to sustain ESC self-renewal. This two-inhibitor combination of CHIR 99021 and PD 0325901, known as 2i, can maintain self-renewal in the absence of feeder cells and exogenous growth factors.

In addition to the known advantages of using small molecules in the stem cell workflow, i.e. they are chemically-defined and cell-permeable, Tamm *et al.* (2013) reported that ESCs grown in 2i medium show lower levels of spontaneous differentiation compared with standard SC culture methods. 2i can also effectively rescue cultures that have started to differentiate and can be used to adapt feeder-dependent mESCs to feeder-free surfaces with little evidence of cell death.

While stem cells can be propagated almost indefinitely in 2i containing medium, they are vulnerable to apoptosis during single cell dissociation in routine passage. Watanabe *et al.* (2007) found that a Rho-associated coiled-coil kinase (ROCK) inhibitor, Y-27632 (Cat. No. 1254), can significantly reduce dissociation-induced apoptosis of ESCs, improving cell survival and colony formation. One-hour pretreatment of hESCs with Y-26732, prior to dissociation and plating on a MEF feeder layer improved cloning efficiency to 26.6% compared with around 1% for untreated cells. Y-27632 treated cells produced many large colonies and retained the ability to grow and differentiate through 30 passages. Other ROCK inhibitors, Fasudil (HA 1077; Cat No. 0541) and Thiazovivin (Cat. No. 3845) have similar effects on cloning efficiency of hESCs, while inhibitors of other kinases are ineffective. The highly potent ROCK inhibitor Chroman 1 (Cat. No. 7163) has also been shown to improve survival of hPSCs.

Research has shown that hESCs exhibit integrin-dependent matrix adhesion and E-cadherin-dependent cell-cell adhesion; single cell dissociation leads to disruption of these interactions resulting in apoptosis. The loss of E-cadherin-dependent intercellular contact leads to hyperactivation of Rho/ROCK signaling. Conversely ROCK inhibition leads to increased E-cadherin levels and cell attachment to the extracellular matrix (ECM). In addition, cells plated onto an E-cadherin-coated plate show decreased Rho activity, indicating that E-cadherin-mediated cell-cell interaction likely regulates Rho/ROCK activity in hESCs. Pyrintegrin (Cat. No. 4978) enhances cell-ECM adhesion-mediated integrin signaling and improves cloning efficiency of hESCs, but has no effect on ROCK.

The peroxisome proliferator-activated receptor γ (PPARγ) activator Pioglitazone (Cat. No. 4124) has been shown to act synergistically to enhance the effects of Y-27632 on dissociation-induced apoptosis, improving cloning efficiency of hESCs and hiPSCs by 2-3-fold compared with ROCK inhibitor alone in feeder-free culture systems. Together Pioglitazone and Y-27632 upregulate E-cadherin and β-catenin, which are downregulated in dissociated stem cells. PPARγ acts via inhibition of GSK-3β (see Stem Cell Signaling) to increase membranous β-catenin levels, which interacts with E-cadherin. Pioglitazone alone has no effect on cloning efficiency.

In addition to preserving the stemness of PSCs, it is also important to develop methods to maintain lineage-restricted or terminally differentiated cells in a differentiated state. This is particularly critical for cell therapy purposes, as differentiated cells are less prone to teratoma formation than PSCs. However long-term maintenance of terminally differentiated cells presents challenges, since differentiated cells often lose their identity and functionality in culture. Maintenance of cell function is regulated by a network of signaling pathways (see also Stem Cell Signaling section) and these need to be recapitulated in vitro to stabilize cells over the long term.

Kyoto Probe-1 (KP1, Cat. No. 7419) is a useful tool for monitoring pluripotency during ESC and iPSC maintenance. This fluorescent probe localizes to mitochondria in undifferentiated iPS/ES cells only and so distinguishes differentiated from undifferentiated cells. It is suitable for use in live cell imaging as well as flow cytometry. Li *et al.* (2011) established chemically-defined conditions for the maintenance of hESC-derived primitive neuroepithelium in vitro (see also Differentiation section) using LIF, CHIR 99021 and the TGF-β receptor inhibitor SB 431542 (Cat. No. 1614). In the presence of this cocktail of reagents, these primitive neural stem cells (pNSCs) self-renew over multiple passages on basement membrane extract (BME; e.g. Cultrex™, available from R&D Systems), maintaining a stable NSC phenotype and retaining the ability to differentiate into midbrain and hindbrain neuronal cell types in response to the appropriate cues.

More recently Chen *et al.* (2021) identified a different cocktail of small molecules, a combination of Chroman 1 (Cat. No. 7163), Emricasan (Cat. No. 7310), Lyophilized Polyamines (Cat. No. 7739), and Trans-ISRIB (Cat. No. 5284) termed CEPT, which improves the viability of hPSCs. This combination

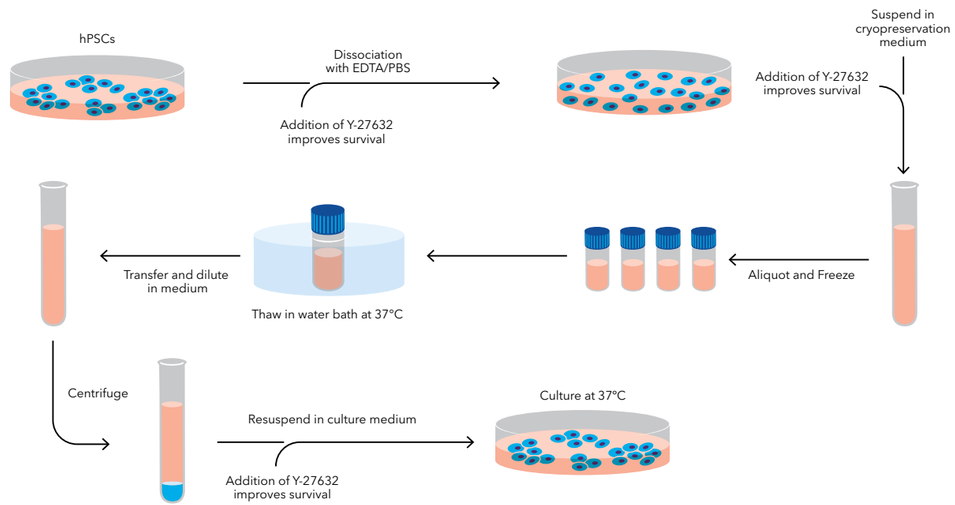


Figure 5. Using ROCK inhibitor Y-27632 to improve cell survival in cryopreservation. From Liu and Chen (2014) Curr. Protoc. Stem Cell Biol. 31:IC.17.1.

DMSO, Cell Cryopreserve Grade

DMSO, Cell Cryopreserve Grade (Catalog # 7726) is a cryoprotective agent that penetrates the cell membrane. It reduces the osmotic charge of the cells during freezing and thawing and reduces the osmotic shock. The product also protects the cells from dehydration and shrinking during freezing and prevents the formation of ice crystals.

also significantly improved cell survival during embryoid body and organoid formation compared with Y-27632 alone.

Hepatocytes are valuable in drug metabolism and toxicity studies, as well as for modeling liver diseases, such as hepatitis B virus (HBV) infections. Xiang *et al.* (2019) found that after 24 hours in culture, primary human hepatocytes (PHH) showed downregulation of genes important in maintaining hepatocyte function and upregulation of epithelial-mesenchymal transition (EMT) inducers. They subsequently investigated a range of small molecules and found that a combination of SB 431542, the adenylate cyclase activator, Forskolin (Cat. No. 1099), Notch inhibitor DAPT (Cat. No. 2634), Wnt inhibitor IWP 2 (Cat. No. 3533), and the BMP inhibitor, LDN 193189 (Cat. No. 6053), suppresses expression of EMT marker genes. This chemical approach, termed the 5C cocktail, maintains the functionality of PHHs over the long term and provides a platform for the study of HBV infection.

Storage/Cryopreservation

The successful use of stem cells for research and stem cell therapy requires efficient storage by cryopreservation. There are two cryopreservation methods, fast and slow freezing, neither of which are efficient. After slow freezing, the survival rate of hESCs and hiPSCs is poor, at around 10%. Improvement in hESC survival post-thaw can be achieved by first treating cells with ROCK inhibitor Y-27632 prior to dissociation as described above, then slow freezing and storage as single cells rather than clumps. Y-27632 added to the cryopreservation medium has also been shown improve post-thaw survival of hESCs cultured on feeder layers (FIGURE 5).

Addition of ROCK inhibitor Y-27632 to the post-thaw culture medium can also improve the viability of cryopreserved hESCs and hiPSCs compared with untreated cells. Treatment with ROCK inhibitor increases the adherent properties of cells post-thaw. Y-27632-treated freeze-thawed hESCs also retain morphology, stable karyotype, expression of cell surface markers, and pluripotency. Y-27632 also decreases recovery time of cells after cryopreservation, producing confluent plates of undifferentiated colonies within 7-10 days. (Martin-Ibanez *et al.*, 2008; Li *et al.* 2008). In addition, the CEPT cocktail, described by Chen *et al.* (2021) was shown to improve survival of differentiated cells following cryopreservation, with cardiomyocyte survival increasing by 36% and motor neuron survival increasing by 63% compared to DMSO controls.

Differentiation

Under the appropriate conditions pluripotent stem cells can be directed to differentiate into almost any specialized cell type. Differentiated cells can be used in a variety of applications, including drug screening, toxicity testing, and disease modeling. This approach also has potential in regenerative medicine for conditions such as neurodegenerative diseases, diabetes, heart failure, and traumatic injury. To generate lineage-restricted cells that can be used in therapy and research, the challenge is to develop a system that is easy, reproducible, rapid and efficient.

Differentiation of stem cells is controlled by numerous signaling pathways, which regulate cellular processes such as gene transcription and changes in chromatin structure. The differentiation of cells towards a specific cell type depends on the activation of signals that promote the generation of required cell type and the inhibition of signals that promote self-renewal or differentiation to unwanted lineages. Chemical approaches using small molecules allow for the precise tuning of these signals, as well as improving the efficiency and rate of differentiation. The most reproducible and efficient methods for differentiating stem cells recapitulate key steps in the development of the early embryo with precise temporal activation and inhibition of relevant signaling pathways.

Neural Differentiation

Key signaling pathways in neuronal differentiation include Wnt, BMP, FGF, and retinoic acid (RA) signaling cascades. The generation of neural progenitors in vitro is characterized by expression of neuroepithelial markers, such as Nestin, PAX6, SOX1, SOX3, PSA-NCAM and MUSASHI-1, and the formation of neural rosettes, reminiscent of neural tube initiation in vivo. Various methods have been developed for the conversion of iPS and ES cells into neuronal lineages,

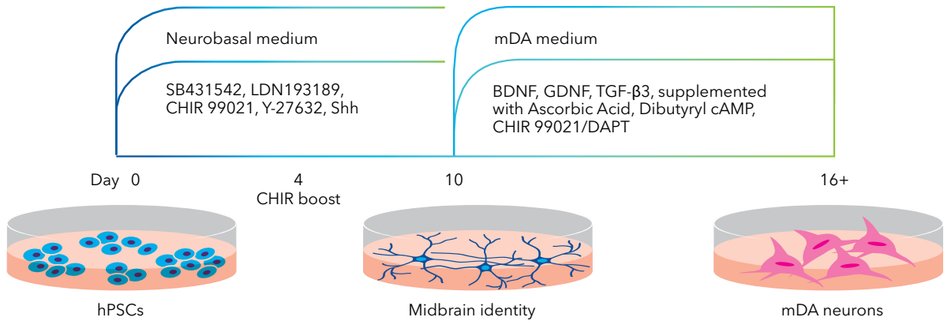


Figure 6. Schematic outlining a protocol for the generation of midbrain dopaminergic neurons from hPSCs. From Kim *et al.* (2021) Cell Stem Cell 28:343.

with a view to improving our understanding of neurological diseases and the neurobiological processes involved in development.

Noggin is an endogenous BMP antagonist and neural-inducing factor. Chambers *et al.* (2009) reported that dual inhibition of SMAD signaling by Noggin, and the small molecule TGF-β antagonist, SB 431542 (Cat. No. 1614), promotes rapid induction of $PAX6^+$ neuroepithelial cells from hESCs, capable of rosette formation. It has subsequently been found that the small molecule ALK2/3 antagonist, LDN 193189 (Cat. No. 6053) can replace Noggin in dual SMAD inhibition for the generation of neural progenitors (NPs). These NPs can be directed to become midbrain floor plate (FP) precursors, characterized by the expression of the marker FOXA2, by exposure to small molecule activators of Hedgehog signaling, such as Purmorphamine (Cat. No. 4551). Activation of Wnt signaling, using the potent GSK-3β inhibitor CHIR 99021 (Cat. No. 4423), promotes the conversion of these FP precursors to a dopamine (DA) neuron fate (Kriks *et al.*, 2011), characterized by the co-expression of FOXA2 and the roof plate marker LMX1A (FIGURE 7). DA neurons have the potential as cell therapy for Parkinson's disease (PD). Researchers at Kyoto University (Kikuchi *et al.*, 2017) have generated DA neuron precursors from hiPSCs derived from healthy donors and patients with PD. Their method uses dual SMAD inhibition by LDN 193189 and the TGF-β inhibitor A 83-01 (Cat. No. 2939) for neuronal induction, followed by induction of floor plate cells with Purmorphamine, CHIR 99021 and FGF-8. They have shown that DA precursors derived in this way survive and function as midbrain DA neurons when transplanted into animal models of PD and that the recipients show increased spontaneous movement following transplantation. The method devised by Kikuchi *et al.* has been used in a clinical trial for Parkinson's disease at Kyoto University.

Li *et al.* (2011) discovered that combined inhibition of GSK-3β, TGF-β, and Notch signaling with CHIR 99021, SB 431542, and the γ-secretase inhibitor Compound E (Cat. No. 6476), respectively, rapidly converts hESCs to primitive neuroepithelium within 1 week. This small molecule cocktail leads to the loss of the pluripotency markers, Oct4 and Nanog with a concurrent increase in PAX6 expression. Expression of Sox2, which is both a pluripotency marker and a marker of neural differentiation, remains unchanged.

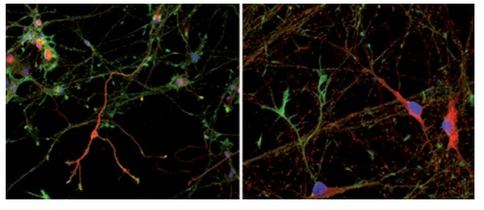


Figure 7. Dopaminergic neurons derived using SB 431542, CHIR 99021, DAPT and Purmorphamine. Cells cultured in Neuronal Media supplemented with TGFβ3 (Cat. No. 8420-B3, R&D Systems), cAMP, GDNF (Cat. No. 212-GD, R&D Systems), BDNF (Cat. No. 248-BDB, R&D Systems), N21-MAX (Cat. No. AR008, R&D Systems). Images courtesy of Kevin Flynn, Bio-Techne.

A paper by Kim *et al.* (2021) describes a protocol that uses biphasic Wnt signaling using a cocktail of small molecules and proteins to derive midbrain dopaminergic (mDA) neurons from human PSCs. Neuronal specification was induced with a combination of the small molecules, SB 431542, LDN 193189, CHIR 99021, and Y-27632, along with Sonic Hedgehog (Shh) protein (R&D Systems, Cat. No. 8908-SH). A boost in the concentration of CHIR 99021 from day 4 of differentiation was found to improve midbrain specification and reduce the presence of unwanted cell types. Differentiation of mDA neurons was achieved by switching to media containing the growth factors BDNF (R&D Systems, Cat. No. 11166-BD), GDNF (R&D Systems, Cat. No. 212-GD) and TGF-ß3 (R&D Systems, Cat. No. 8420-B3), supplemented with Ascorbic acid (Cat. No. 4055), Dibutyryl-cAMP (Cat. No. 1141), and CHIR 99021 from day 10. Substitution of CHIR for DAPT (Cat. No. 2634) occurred in the latter stages of differentiation. The resulting cells were found to reproducibly exhibit the hallmarks of midbrain dopaminergic (mDA) neurons. When transplanted into the striatum in a hemiparkinsonian rat model, differentiated mDA neurons exhibited long-term survival and animals showed functional recovery, as indicated by reduced amphetamine-induced rotational behavior. Importantly from a regenerative medicines standpoint, the cells remained functional after cryopreservation and thawing.

This protocol has been adapted to generate mDA neurons (DA01) for use in a clinical study in Parkinson's disease patients, which began in 2021.

In the context of stem cell therapy for regenerative medicine, PluriSIn 1 (Cat. No. 4847) could be a useful tool as it selectively eliminates undifferentiated hPSCs from culture.

Cardiomyocyte Differentiation

Stem cell-derived cardiomyocytes (CM) have multiple potential uses in disease modeling and therapy. Signaling pathways important in the control of CM differentiation from PSCs, include BMP, Wnt, and TGF-β. Two distinct methods for the cardiac differentiation of hPSCs have been developed: the formation of embryoid bodies (EBs), and the culturing of hPSCs as a monolayer. The EB methodology involves suspending hPSC colonies in media to form spherical aggregates and produces >70% CM but is complex and time consuming. The monolayer-based method for cardiac differentiation of hPSCs produces a higher yield (>85%) and is easier to use.

Lian *et al.* (2012) investigated the role of Wnt/β-catenin signaling in cardiac induction of stem cells using both the EB and monolayer techniques. They showed that the use of a small molecule, the GSK-3β inhibitor CHIR 99021, to activate the Wnt/β-catenin pathway is sufficient to drive hPSCs to differentiate into CM under fully defined, growth factor-free conditions in vitro. In addition, treatment of human PSCs with CHIR 99021 followed by the PORCN inhibitors IWP 2 (Cat. No. 3533) or IWP 4 (Cat. No. 5214) to inhibit Wnt signaling, resulted in the generation of spontaneously contracting cardiomyocytes exhibiting normal sarcomere organization and a predominantly ventricular-like action potential. Their findings suggest that canonical Wnt signaling likely acts as a master regulator of CM specification and that the precise temporal modulation of signaling is important in the determination of cardiac fate.

BIO (Cat. No. 3194) is another GSK-3β inhibitor that has been found to promote differentiation of CM from hPSCs. Minami *et al.* (2012) investigated the derivation of CM from a range of hPSC lines in monolayer culture under defined cytokine- and serum-free conditions. Activation of Wnt signaling using a combination of CHIR 99021 plus BIO during days 0-3 of cardiac differentiation, followed by Wnt signaling inhibition using KY 02111 (Cat. No. 4731) and the tankyrase inhibitor XAV 939 from day 3 of differentiation onwards, resulted in the emergence of beating colonies by around day 8-10. This protocol was highly efficient, with up to 98% of resulting cells staining positive for the cardiac marker cardiac troponin T (cTnT), and having well organized sarcomeres and electrophysiological characteristics consistent with CM.

The efficiency of specific CM differentiation protocols shows considerable variability between cells lines. Qiu *et al.* (2017) carried out a screen for small molecules that promote cardiac differentiation of stem cells and identified the mTOR (mammalian target of rapamycin) inhibitor Rapamycin (Cat. No. 1292) as a promoter of cardiomyocyte differentiation.

The researchers found that when they used Rapamycin + CHIR 99021 in place of BIO + CHIR 99021 in the early stages of CM differentiation, efficiency of CM generation was maintained across different human hESC and hiPSC lines. The addition of Rapamycin inhibits the apoptosis of hESCs in high-density monolayer culture and promotes mesoderm formation and the research highlights mTOR as an important regulator of cardiogenesis.

Other compounds have been found to enhance CM differentiation, such as T3 (triiodothyronine; Cat. No. 6666), which promotes maturation of hiPSC-derived CMs, increasing CM size and sarcomere length and improving contractile kinetics. Poly(I:C) (polyinosinic-polycytidylic acid; Cat. No. 4287) has also been shown to accelerate CM maturation when used to prime early cardiac progenitors differentiated from hiPSCs and hESCs in monolayer differentiation protocols using small molecule modulation of Wnt signaling. Epigenetic and transcriptional profiling of primed cardiac progenitor cells reveals increased histone acetylation and activation of epigenetic marks at promoters of cardiac myofilament genes.

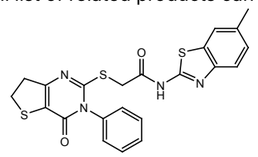
A paper by Noor *et al.* (2019) has demonstrated how cardiac cells differentiated from iPSCs might be used in regenerative medicine (FIGURE 8). The researchers took omental (peritoneal) tissue biopsies from patients and separated the cellular and acellular materials. A personalized hydrogel was produced from the extracellular matrix, while the cells were first reprogrammed to iPSCs, then differentiated into endothelial cells and cardiomyocytes. The two different cell types were separately combined with the hydrogel to form two "bioinks", which were used to generate patient-matched, thick, vascularized and perfusable cardiac patches by 3D printing. This approach has potential for engineering personalized tissues for transplantation, eliminating the need for immunosuppression. The technique was also used to generate heart-like structures, and the researchers anticipate that it could eventually lead to the production of human hearts for transplantation.

β-Cell Differentiation

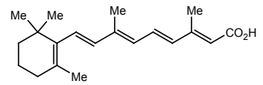
In the developing embryo, the pancreas develops from the definitive endoderm (DE). The formation of pancreatic progenitors from DE is dependent on activation of retinoid and BMP signaling and inhibition of Hedgehog signaling. Pancreatic progenitor cells are characterized by the expression of the transcription factor PDX1, and give rise to endocrine, exocrine, and ductal cells. FGF signaling also has an important role at a later stage of pancreatic cell differentiation.

A method to generate functional human pancreatic β-cells from hPSCs designed by Pagliuca *et al.* (2014) presents possibilities for studying and treating diabetes. Their multi-step protocol uses a combination of 11 small molecules and proteins in a suspension-

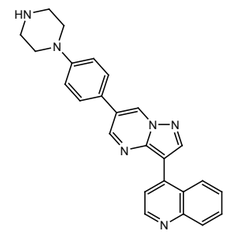
BOX 3: Differentiation Products
A full list of related products can be found on pages 28-36.



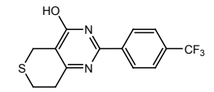
IWP 2 (3533)
Promotes cardiomyocyte differentiation from hPSCs; inhibitor of Wnt processing and secretion



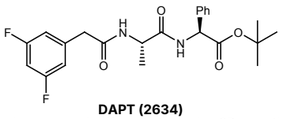
Retinoic Acid (0695)
Promotes differentiation of mouse ESCs into adipocytes, pancreatic cells, neurons, and glia; endogenous agonist for retinoic acid receptors



LDN 193189 (6053)
Promotes neural induction of hPSCs; ALK2/3 inhibitor.
GMP version available



XAV 939 (3748)
Promotes cardiomyocyte differentiation from ESCs; tankyrase inhibitor; inhibits Wnt signaling.
GMP version available



DAPT (2634)
Induces neural differentiation; also 3D growth matrix component; γ-secretase inhibitor

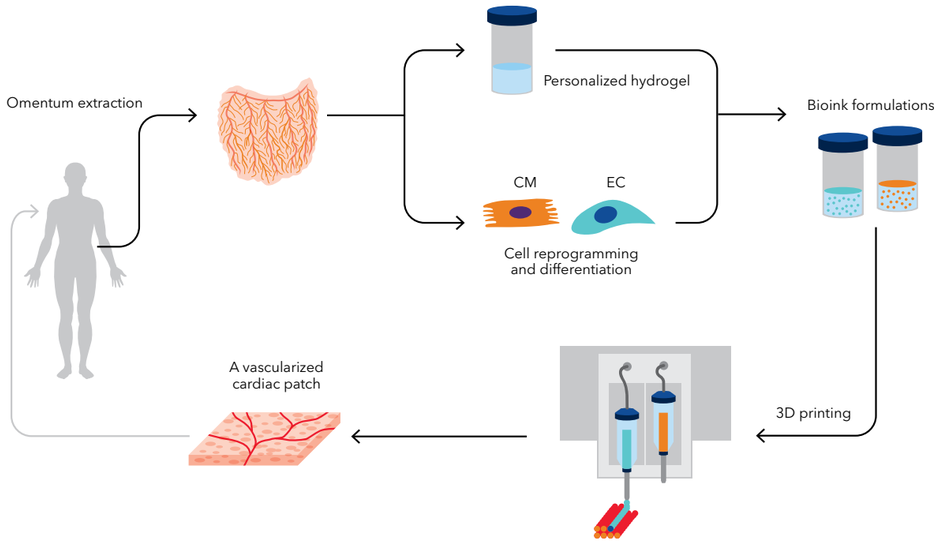


Figure 8. Schematic outlining the generation of autologous tissue patch for regenerative medicine. Omental tissue is extracted from the patient. The cells are separated from the matrix; the former are reprogrammed to PSCs then differentiated to cardiomyocytes (CM) and endothelial cells (EC), while the latter is processed into a hydrogel. The two differentiated cell types are separately encapsulated within the hydrogel to generate the two bioinks used for printing. The bioinks are then printed to engineer vascularized and cellularized structures. Adapted from Noor *et al.* (2019) Advanced Science 6:1900344.

based culture system. The first stage involves the induction of DE using Activin A (R&D Systems: 338-AC) and CHIR 99021. Pancreatic specification is triggered by the addition of Retinoic acid (Cat. No 0695), Keratinocyte Growth Factor (KGF, R&D Systems: 5028-KG) to activate FGF signaling, SANT-1 (Cat. No. 1974) and LDN 193189 to inhibit Hedgehog and BMP signaling respectively, and the protein kinase C (PKC) activator Phorbol 12,13-dibutyrate (Cat. No. 4153). The researchers then screened a range of small molecules affecting signaling pathways to determine the optimum combination for β-cell differentiation from pancreatic progenitors and found that a combination of T3, the γ-secretase inhibitor Compound E, the TGF-β inhibitor RepSox (Cat. No. 3742), plus Heparin (Cat. No. 2812) and Betacellulin (R&D Systems: 261-CE) produces functional β-cells by around day 28 of culture.

These stem cell-derived β-cells resemble primary human β-cells in that they express c-peptide and the nuclear proteins PDX1 and NKX6.1. They also release insulin in response to glucose challenge and, when transplanted into diabetic mice, are found to ameliorate hyperglycemia.

Transdifferentiation

Cells can also be reprogrammed directly from one specialized cell type to another, without first being converted to ciPSCs, a process known as transdifferentiation or direct lineage reprogramming. This technique offers an alternative approach for generating cells for cell therapy and research purposes. Transdifferentiation has the advantage that the starting material, i.e. mature somatic cells such as fibroblasts, is readily available. In addition, since the process does not involve the cells entering an induced pluripotent state, the possibility of tumorigenesis is reduced. The production of lineage-specific cells via transdifferentiation therefore has enormous potential in medicine to replace lost or damaged cells, for example following myocardial infarction or cartilage injury, or in neurodegenerative diseases.

Transdifferentiation can be achieved through introduction of exogenous transcription factors via retroviral transduction, but as with reprogramming, this method of converting cells is slow and inefficient. Other methods include activation or silencing of endogenous genes using techniques such as CRISPR/Cas9 or via pharmacological manipulation of the epigenetic environment and signaling pathways using combinations of small molecules. Transdifferentiation has been used to convert fibroblasts into a wide range of different cell types including NSCs, functional neurons, cardiomyocytes, endothelial cells, hepatocytes, skeletal muscle cells, and pancreatic β cells. Cao *et al.* (2016) described a method to convert human fibroblasts into CMs using a cocktail of nine small molecules (9C). The 9C-treated cells were subsequently cultured in cardiac induction medium and transplanted into mice, where they converted into CM-like cells. The 9C cocktail comprises CHIR 99021, A 83-01 (Cat. No. 2939), Pluripotin, OAC-2, Y-27632 (Cat. No. 1254), BIX 01294 (Cat. No. 3364), AS 8351, SU 16f (Cat. No. 3304) and JNJ 10198409 (Cat. No. 6976). This technique is highly efficient, with around a 97% conversion rate from fibroblasts to spontaneously beating ciCM in around 20 days. When transplanted into mice with infarcted hearts, 9C-treated fibroblasts were efficiently converted into cardiomyocyte-like cells. (FIGURE 9).

A protocol for the transdifferentiation of functional neurons from mouse fibroblasts using small molecules has been reported by Li *et al.* (2015). The method uses a combination of four reagents, Forskolin (Cat. No. 1099), ISX 9 (Cat. No. 4439), CHIR 99021 and I-BET 151 (Cat. No. 4650) and results in an approximately 90% conversion rate after 16-20 days induction. Resulting cells express multiple neuron-specific markers and have extensive neurite outgrowth. Co-culture of these induced cells with astrocytes in maturation medium for a further 14-21 days resulted in functional neurons capable of generating action potentials and forming functional synapses. In this study, the researchers found that the BET bromodomain inhibitor I-BET 151 was necessary in the protocol to suppress endogenous fibroblast fate-determining programs by disrupting the fibroblast core transcriptional network. ISX 9, on the other hand, is essential for the induction of master neural genes.

A group from the Huck Institute of Life Sciences (Yin *et al.*, 2019) have identified a protocol for generating neurons from astrocytes. The researchers found that simultaneously inhibiting GSK-3β, and the Notch, TGFβ, and BMP pathways with CHIR 99021, DAPT, SB431542, and LDN 193189, respectively, is sufficient to convert human astrocytes into neurons. These chemically-induced neurons also fire action potentials and survive in the long-term in culture. The findings of this work are significant in that the researchers found that direct intracranial administration of this combination of compounds in adult mice can induce hippocampal neurogenesis, which has possible implications for the future treatment of neurodegenerative diseases.

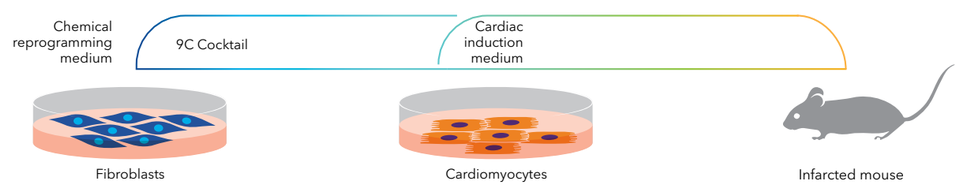


Figure 9. Schematic outlining a protocol to transdifferentiate fibroblasts into cardiomyocytes. From Cao *et al.* (2016) Science 352:1216.

Organoids

Organoids are 3D tissue/organ models made up of pluripotent stem cells and other supporting co-cultured cells such as epithelial cells. When cultivated appropriately, these stem cells have the ability to self-organize into organ-like tissue exhibiting some functions of the organ it is modeling. Organoids make stable model systems and are amenable to long-term cultivation.

Organoids can be derived from human stem cells or from patient-derived iPSCs. They self-organize through cell sorting (FIGURE 10), with different cell types arranging themselves based on the distinct expression profiles of cellular adhesion molecules and spatially restricted lineage commitment. Spatially constraining cells in tissue or artificial conditions promotes further differentiation of stem cells and is crucial in the generation of organoids. In the laboratory, lineage commitment is most commonly encouraged using the biological scaffolds derived from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells (i.e. Cultrex™ Basement Membrane Extracts). These scaffolds provide environmental cues, such as growth factors, which encourage cells to attach and form organoid structures.

Organoids have three defining characteristics:

- They consist of multiple cell types found in the organ they are modeling
- They exhibit some of the functionality of the organ they are modeling
- The organoid cells must be organized in a similar manner to the organ they are modeling

Human inducible pluripotent stem cells (hiPSCs) derived from patients with diseases such as cardiomyopathy and Parkinson's disease have been cultivated into organoids, providing the most relevant model systems and enabling the interrogation of the mechanisms underlying disease states. In addition to disease modeling, organoids are useful research tools in developmental biology, drug discovery, and toxicology screening. They also have potential in drug screening for personalized medicine as well as organ replacement therapy.

Many types of organoids, including brain, pancreas, heart, lung, intestine, liver, optic cup, and cancer, have been developed to date. Small molecules are increasingly being used to grow and maintain organoids because of their ease of use, controllable production methods, and quality. In addition, they are highly chemically defined with low lot variability and high purity, making results consistently repeatable.

Lancaster *et al.* (2013) developed a reproducible hPSC-derived 3D culture system for the generation of cerebral organoids. These organoids have complex heterogeneous tissues and develop distinct regions with mature cortical neuron subtypes reminiscent of the developing human brain by day 20-30. These organoids lack vascularization, however, which limits their size (FIGURE 11).

Lancaster *et al.* showed how this method might be used to model neurological diseases. They grew organoids from hiPSCs derived from skin fibroblasts of patients with microcephaly. EBs grown using these conditions were smaller than those grown from normal hESCs and failed to develop when transferred to neural induction media. Modification of the protocol by increasing the initial number of iPSCs allowed the development of neuroectoderm, but neural tissues were smaller overall and contained fewer progenitor regions. Based on their findings, the researchers concluded that the smaller brain size associated with microcephaly may be the result of premature neural differentiation and a failure of the radial glial progenitor cell population to expand.

A protocol to cultivate kidney organoids from hESCs or hiPSCs in around 25 days has been established by Takasato *et al.* (2016). The method requires the initial culture of monolayers of stem cells in MEF-conditioned ESC medium. The medium is then switched to an animal product-free medium (APEL or Albumin Polyvinylalcohol Essential Lipids) supplemented with CHIR 99021 (Cat. No. 4423) for induction of intermediate mesoderm. After 2 to 5 days, the CHIR 99021 is removed and exchanged for FGF-9 (R&D Systems; Cat. No. 273-F9) plus Heparin (Cat. No. 2812). On day 7, cells are harvested and aggregated for 3D culture to facilitate organoid formation; growth factors are withdrawn around day 12. Nephron, ureteric epithelial, renal interstitial, and endothelial progenitors are formed which self-organize and form kidney organoids by day 25. This protocol recapitulates the developmental process of human kidney organogenesis, and organoids generated using this technique have the potential for modeling renal diseases and screening for nephrotoxic drugs.

Crespo *et al.* (2017) generated colonic organoids (CO) from human ESCs and iPSCs under chemically-defined conditions. Similar to the generation of pancreatic β-cells (see Differentiation section), the first step is the generation of definitive endoderm (DE) by culturing hESCs in CHIR 99021 and Activin A (R&D Systems; Cat. No. 338-AC) for 4 days. This is followed by a further 4 days in the presence of CHIR 99021 plus FGF-4 (R&D Systems; Cat. No. 235-F4) to direct cell differentiation towards hindgut endoderm (HE). Cells are cultured for a further 12 days with CHIR 99021, LDN 193189 (Cat. No. 6053) and EGF (R&D Systems; Cat. No. 236-EG) to generate colonic epithelial cells. Following dissociation of cells and embedding in Matrigel beads embryonic gut-like spheroids are formed, which then cavitate into fully convoluted colonic organoids. The researchers used this technique to generate COs from hiPSCs derived from patients with familial adenomatous polyposis, which were used as a drug testing platform to identify potential therapies for this condition.

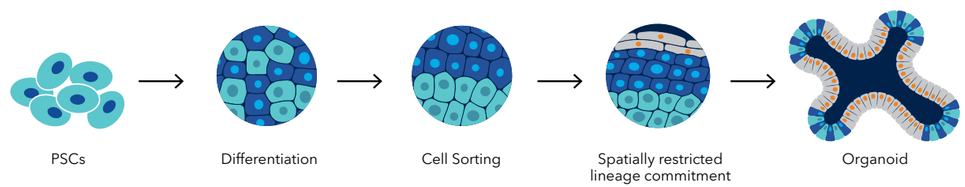


Figure 10. Stages of organoid genesis.

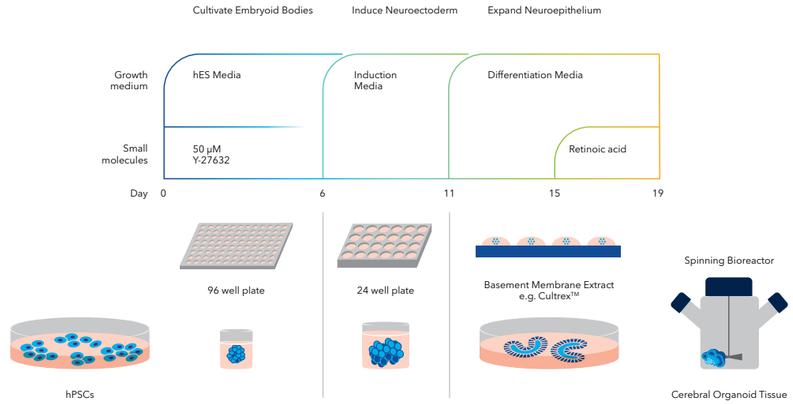


Figure 11. Snapshot of cerebral organoid genesis protocol. In brief embryoid bodies are cultivated in hES media supplemented with bFGF and Y-27632 (Cat. No. 1254). On Day 6, cells are transferred to 24 well plates and grown in induction media. On Day 11, tissue is transferred to droplets of basement membrane extract (e.g. Cultrex™) then grown in differentiation media. After 4 days of stationary growth, the tissue droplets are moved to a spinning bioreactor and grown in differentiation media containing Retinoic acid (RA; Cat. No. 0695). Adapted from Lancaster *et al.* (2013). Nature 501:373. For more organoid protocols, visit rndsystems.com/protocol-types/organoid-and-3-d-cell-culture-protocols.

GMP and Ancillary Material Grade Small Molecules

To support the development of new cell therapies we have created a range of enhanced quality products for use as ancillary reagents (also known as raw materials). Manufactured in our ISO 9001 certified facilities using strictly controlled processes, and with rigorous quality management systems, our GMP and Ancillary Material Grade compounds are intended for use in the manufacture of cell and gene therapies.

Ancillary Materials Qualification

The term ancillary reagent refers to those components used in the manufacture of cell and gene therapies or tissue-engineered therapies, such as small molecules, proteins, cell culture media etc, which are not intended to be part of the final product. The quality of these ancillary reagents can impact the safety, purity, and thus suitability of the final cell product for clinical use. Small molecules offer several advantages when compared to other ancillary materials for cell therapy manufacture as outlined on page 3 of this Product Guide.

It is important to assess the source, purity, identity, safety, and suitability of your ancillary materials. If using an ancillary reagent in the development of a cell therapy where these factors are unknown, extensive qualification studies may be required by medicine regulators to ensure that the material used is suitable and will not compromise the safety of the final cell therapy product. Our enhanced quality small molecule ranges, GMP and Ancillary Material (AM)-Grade have been developed with cell and gene therapy manufacturing in mind so they are produced with a higher level of scrutiny, documentation, and quality control than our standard catalog products.

What are Ancillary Material Grade Compounds?

The production of our Ancillary Material Grade compounds is in accordance with ISO TS 20399 guidelines. The synthesis of Ancillary Material Grade small molecules uses established synthetic procedures allowing a wide range of products to be manufactured to the superior AM-grade rather than just focusing on the highly risk-assessed and time-consuming GMP process.

The key differences compared with our standard catalog (research use only or RUO) products include enhanced QC testing, a more detailed QA review and accompanying documentation. AM-grade products are manufactured to provide:

- Traceability of starting materials
- Animal-free manufacturing process (TSE and BSE Certification)
- Segregated manufacturing area and rigorous cleaning procedures to minimize cross contamination risk
- Final product weighing using aseptic techniques in an ISO 7 cleanroom
- Enhanced quality control including Bioburden and Endotoxin testing on final products

What are GMP Compounds?

Our GMP (Good Manufacturing Practice) small molecules are produced according to Current Good Manufacturing Practice guidelines. In addition to the controls listed above, the following also apply to our GMP compounds:

- The manufacturing process follows the relevant sections of ICH Q7 guidelines (Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients).
- The synthetic routes for these small molecules are risk assessed in depth at every step and products are subject to additional analysis.
- Our GMP small molecules are classified tier 2 risk, as per USP <1043>: Ancillary Materials for Cell, Gene and Tissue-Engineered Products.

The table below summarizes the quality attributes of the different product ranges available from Tocris.

Tocris Product Quality Attributes

	Standard Catalog	Ancillary Material Grade	GMP
ISO 9001	√	√	√
QC Testing	√	√	√
Controlled Manufacturing Zone		√	√
ISO-7 Cleanroom		√	√
Endotoxin/Bioburden Testing		√	√
TSE and BSE Certification		√	√
Follow ISO 20399:2022 Guidelines		√	√
Risk-based Approach to Manufacturing			√
ICH Q7			√

Tocris Bioscience and R&D Systems, part of the Bio-Techne family of brands, offer innovative solutions for your stem cell therapy manufacturing needs, with a portfolio that includes off-the-shelf GMP small molecules, the largest range of GMP proteins, as well as GMP media and media supplements.

Tocris Small Molecules for Stem Cell Research

Ancillary Material Grade			
	Product Name	**Catalog #**	**Primary Action**
	A 83-01	TB2939-RMU	A 83-01 synthesized to Ancillary Material Grade
	L-Ascorbic acid	TB4055-RMU	L-Ascorbic acid synthesized to Ancillary Material Grade
	DAPT	TB2634-RMU	DAPT synthesized to Ancillary Material Grade
	Dexamethasone	TB1126-RMU	Dexamethasone synthesized to Ancillary Material Grade
	DZNep HCl	TB4703-RMU	DZNep synthesized to Ancillary Material Grade
	endo-IWR 1	TB3532-RMU	*endo*-IWR 1 synthesized to Ancillary Material Grade
	Forskolin	TB1099-RMU	Forskolin synthesized to Ancillary Material Grade
	Nicotinamide	TB4106-RMU	Nicotinamide synthesized to Ancillary Material Grade
	RepSox	TB3742-RMU	RepSox synthesized to Ancillary Material Grade
	Retinoic acid	TB0695-RMU	Retinoic acid synthesized to Ancillary Material Grade
	Staurosporine	TB1285-RMU	Staurosporine synthesized to Ancillary Material Grade
	T3	TB6666-RMU	T3 synthesized to Ancillary Material Grade
	TCS JNK 6o	TB3222-RMU	TCS JNK 6o synthesized to Ancillary Material Grade
CEPT Cocktail			
	Chroman 1	7163	Highly potent and selective ROCK inhibitor, maintains survival of hPSCs
	Emricasan	7310	Potent pan-caspase inhibitor, promotes survival of hPSCs
	Polyamine Supplement x1000 (lyophilized)	7739	Media supplement to boost cell growth; component of CEPT cocktail to enhance stem cell viability
	Trans-ISRIB	5284	Integrated stress response (ISR) inhibitor; promotes survival of hPSCs
	CEPT Cocktail Kit	7991	Cell culture supplement for improving stem cell survival
Cryopreservation			
	DMSO, Cell Cryopreserve Grade	7726	Intracellular cryoprotective agent
Differentiation			
β Cell	Compound E	6476	Used in the generation of β cells from hPSCs; γ-secretase inhibitor
	ISX 9	4439	Used in protocols to generate β cells
	RepSox	3742	Used in protocols to generate β cells from hPSCs; TGF-β RI inhibitor
	SANT-1	1974	Used in protocols to generate β cells from hPSCs; TGF-β RI inhibitor; inhibits Hedgehog signaling
	TPPB	5343	Induces differentiation of hESCs into pancreatic progenitors; PKC activator
	T3	6666	Used in the generation of β cells
	Wnt-C59	5148	Induces differentiation of β cells; PORCN inhibitor
Cardio-myocyte	5-Azacytidine	3842	DNA methyltransferase inhibitor; induces differentiation of MSCs into cardiomyocytes
	Dorsomorphin dihydrochloride	3093	Potent AMPK inhibitor; also BMP type I receptor inhibitor; promotes cardiomyocyte differentiation in mouse ESCs
	1-EBIO	1041	Activator of epithelial K_{ca} channels; promotes differentiation of ESCs into cardiomyocytes
	ISX 9	4439	Induces cardiomyogenic differentiation; neurogenic agent; induces neuronal differentiation of SVZ progenitors

Differentiation			
	Product Name	**Catalog #**	**Primary Action**
Cardio-myocyte	IWP 2	3533	PORCN inhibitor; inhibits Wnt processing and secretion; suppresses self-renewal in R1 ESCs and promotes cardiomyocyte differentiation
	IWP 4	5214	Potent inhibitor of Wnt/β-Catenin signaling; induces cardiomyocyte differentiation of human ESCs and iPSCs
	JNJ 10198409	6976	Potent PDGFRα and PDGFRβ inhibitor; enhances transdifferentiation of human fibroblasts into functional cardiomyocytes as part of 9C cocktail
	KY 02111	4731	Inhibits canonical Wnt signaling; promotes differentiation of human ESCs and iPSCs into cardiomyocytes
	Poly (I:C)	4287	TLR3 agonist; promotes maturation of hPSC-derived cardiomyocytes
	Pyridone 6	6577	Potent pan-JAK inhibitor; induces intermediate mesoderm; cell-permeable
	SU 16f	3304	Potent and selective PDGFRβ inhibitor; component of 9C cocktail for conversion of fibroblasts to cardiomyocytes
	T3	6666	Thyroid hormone; promotes differentiation and maturation of hPSC-derived cardiomyocytes
	T 112	7690	Estrogen-related receptor γ agonist; promotes maturation of hiPSC-derived cardiomyocytes
	Wnt-C59	5148	Highly potent PORCN inhibitor; induces differentiation of iPSCs to cardiomyocytes
	XAV 939	3748	Potent tankyrase inhibitor; promotes cardiomyogenesis
	Zebularine	2293	DNA methyltransferase and cytidine deaminase inhibitor; induces cardiomyocyte differentiation in MSCs
Hepatocyte	Sodium butyrate	3850	Histone deacetylase inhibitor; directs differentiation of mESCs into hepatocytes
Mesen-chymal	2-Phospho-L-ascorbic acid	5778	Ascorbic acid derivative; maintains differentation potential in bone marrow-derived MSCs
	AICAR	2840	AMPK activator; prmotes osteogenic differentiation of bone marrow-derived MSCs
	Dexamethasone	1126	Anti-inflammatory gluococorticoid; induces differentiation of human MSCs
	Kartogenin	4513	Potently induces chondrogenesis in MSCs
	KI-7	6787	Positive allosteric modulator of A_{2B} receptors; potentiates osteoblast differentiation from MSCs
	Nicotinamide	4106	PARP-1 inhibitor; promotes MSC differentiation
	Purmorphamine	4551	Smo receptor agonist
	SK216	6187	Plasminogen activator inhibitor-1 (PAI-1); attenuates TGF-β-dependent epithelial-mesenchymal transition
Neural	Compound E	6476	γ-secretase inhibitor; induces neuronal differentiation
	DAPT	2634	γ-secretase inhibitor; induces neuronal differentiation
	Dibutyryl-cAMP, sodium salt	1141	Cell-permeable cAMP analog; promotes differentiation of hPSCs to dopaminergic neurons
	EC 23	4011	Synthetic retinoid; induces neural differentiation of stem cells
	Fluoxetine hydrochloride	0927	5-HT reuptake inhibitor; induces differentiation of neuronal precursors
	Forskolin	1099	Adenylyl cyclase activator; induces neuronal differentiation
	Hh-Ag1.5	7807	Potent and high affinity Smo receptor agonist; induces differentiation of PSCs into spinal motor and sensory neurons

Differentiation

	Product Name	Catalog #	Primary Action
Neural	IBMX	2845	PDE inhibitor (non-selective); facilitates differentiation of neural progenitor cells
	ISX 9	4439	Neurogenic agent; induces neuronal differentiation of SVZ progenitors and also induces cardiomyogenic differentiation
	KHS 101 hydrochloride	4888	Selective inducer of neuronal differentiation in hippocampal neural progenitors
	LDN 193189	6053	Potent and selective ALK2 and ALK3 inhibitor; promotes neural induction of hPSCs
	Metformin	2864	Activator of LKB1/AMPK; enhances neurogenesis; antidiabetic agent
	ML 184	6668	Selective GPR55 agonist; also promotes NSC proliferation and differentiation
	1-Oleoyl lysophosphatidic acid sodium salt	3854	Endogenous agonist of LPA_1 and LPA_2; inhibits differentiation of neural stem cells into neurons
	P7C3	4076	NAMPT activator; also proneurogenic and neuroprotective
	PD 173074	3044	FGFR1 and -3 inhibitor; inhibits proliferation and differentiation of oligodendrocyte progenitors
	PNU 74654	3534	β-Catenin binder; inhibits Wnt signaling; promotes neural differentiation of hPSCs as part of a chemical cocktail
	SAG	4366	Potent Smoothened receptor agonist; activates the Hedgehog signaling pathway; enhances neuronal differentiation of iPSCs into dopaminergic neurons
	SAG dihydrochloride	6390	Dihydrochloride salt of SAG
	SB 431542	1614	Promotes neural differentiation of hPSCs; inhibitor of TGF-β RI, AKL4, and ALK7
	TWS 119	7405	GSK-3β inhibitor; induces neuronal differentiation of ESCs
Osteogenic	CW 008	5495	PKA signaling activator; promotes osteogenesis from hMSCs
Retinal	CKI 7 dihydrochloride	5329	CK1 inhibitor; induces retinal cell differentiation from human ESCs and iPSCs
	EC 23	4011	Synthetic retinoid; induces neural differentiation of hESCs
	Retinoic acid	0695	Promotes maturation of retinal organoids; retinoic acid receptor agonist
Other Different-iation Products	Clemastine fumarate	1453	H_1 antagonist; promotes differentiation of oligodendrocytes from progenitors
	FM19G11	6653	HIF α subunit inhibitor; inhibits transcriptional activity of pluripotency markers
	Ketoconazole	1103	Cytochrome P450c17 inhibitor; promotes differentiation of oligodendrocytes from progenitors
	NNMTi	6900	Nicotinamide N-methyltransferase (NNMT) inhibitor; prmotes myoblast differentiation
	PluriSIn 1	4847	SCD-1 inhibitor; selectively eliminates undifferentiated hPSCs from culture
	Rosiglitazone	5325	Potent and selective PPARγ agonist; promotes differentiation of adipocytes
	SIS3	5291	Selective Smad3 inhibitor; inhibits TGF-β RI signaling and inhibits TGF-β-induced myofibroblast differentiation
	TPPB	5343	High affinity PKC activator; induces differentiation of hESC to pancreatic progenitors

Epigenetics

	Product Name	Catalog #	Primary Action
Bromo-domains	(+)-JQ1	4499	Potent and selective BET bromodomain inhibitor; cell permeable; promotes reprogramming of fibroblasts
	(-)-JQ1	5603	Negative control for (+)-JQ1
	CPI 203	5331	BET bromodomain inhibitor; arrests cell cycle at G1 phase; promotes reprogramming of fibroblasts to hiPSCs
	I-BET 151 dihydrochloride	4650	BET bromodomain inhibitor; also promotes differentiation of hiPSCs into megakaryocytes
	Lin28 1632	6068	Bromodomain inhibitor; promotes mESC differentiation; also RNA binding protein Lin28 inhibitor
DNA Methyl-trans-ferases	5-Azacytidine	3842	DNA methyltransferase inhibitor; enhancs efficiency of somatic cell reprogramming
	Decitabine	2624	DNA methyltransferase inhibitor; demethylates differentiation related genes
	RG 108	3295	Non-nucleoside DNA methyltransferase inhibitor; enhances efficiency of somatic cell reprogramming
	Zebularine	2293	DNA methyltransferase and cytidine deaminase inhibitor; potentiates differentiation of mesenchymal stem cells to cardiomyocytes
Histone Acetyl-trans-ferases	Anacardic acid	3084	Histone acetyltransferase (HAT) inhibitor; promotes cardiomyocyte differentiation from mESCs
Histone Deace-tylases	MC 1742	5727	Potent class I and IIb HDAC inhibitor
	SAHA	4652	Class I and II HDAC inhibitor; improves efficiency of reprogramming
	Sodium butyrate	3850	Histone deacetylase inhibitor; improves efficiency of reprogramming
	Trichostatin A	1406	Potent histone deacetylase inhibitor; improves efficiency of reprogramming
	Valproic acid, sodium salt	2815	Histone deacetylase inhibitor; improves efficiency of reprogramming
Lysine Methyl-trans-ferases	3-Deazaneplanocin A	4703	EZH2 histone methyltransferase inhibitor; widely used in chemical reprogramming protocols
	BIX 01294	3364	GLP and G9a inhibitor; potentiates induction of iPSCs
	EPZ 00477	5567	Highly potent DOT1L inhibitor; improves reprogramming efficiency

GMP

	CHIR 99021	TB4423-GMP	CHIR99021 synthesized to cGMP guidelines
	LDN 193189	TB6053-GMP	LDN193189 synthesized to cGMP guidelines
	SB 431542	TB1614-GMP	SB431542 synthesized to cGMP guidelines
	XAV 939	TB3748-GMP	XAV939 synthesized to cGMP guidelines
	Y-27632	TB1254-GMP	Y27632 synthesized to cGMP guidelines

Hematopoietic Stem Cells

Product Name	Catalog #	Primary Action
N-Acetylcysteine amide	5619	GSH precursor; antioxidant; maintains HSC function in culture
Alexidine dihydrochloride	3979	Inhibitor of PTPMT1; preserves functional hematopoietic stem cells *ex vivo*
AMD 3100 octahydrochloride	3299	Promotes HSC mobilization and expansion
BIO 5192	5051	Highly potent and selective inhibitor of integrin 41 (VLA-4); promotes HSC and progenitor mobilization
BOP	6047	α9β1/α4β1 integrin inhibitor; promotes HSC mobilization
BOP-JF646	6997	Red fluorescent dual α9β1/α4β1 inhibitor; comprising BOP conjugated to Janelia Fluor® 646; fluorogenic; photostable
CASIN	5050	Cdc42 inhibitor; induces HSC mobilization; restores cell polarity
CH 223191	3858	Potent aryl hydrocarbon receptor (AhR) antagonist, HSC expansion *in vitro*
CPI 203	5331	BET bromodomain inhibitor; promotes HSC expansion
16, 16-Dimethyl Prostaglandin E_2	4027	Synthetic prostaglandin E2 (Catalog # 2296) derivative; regulates HSC development
DiD perchlorate	5702	HSC stain; lipophilic fluorescent reagent
Diprotin A	6019	Dipeptidyl peptidase IV (DPP-IV) inhibitor; enhances HSC viability after harvesting
FICZ	5304	Aryl hydrocarbon receptor (AhR) agonist; facilitates hPSC to HSC manufacture *in vitro*
GW 9662	1508	PPARγ antagonist; promotes HSC expansion; increases HSC engraftment
MB 05032	6618	Potent FBPase inhibitor; promotes HSC expansion
Nicotinamide	4106	PARP1 inhibitor; promotes proliferation and expansion of HSC *in vitro*
Prostaglandin E_2	2296	Endogenous prostaglandin; promotes HSC expansion; increases HSC engraftment
Sildenafil citrate	3784	Promotes HSC mobilization
StemRegenin 1	7086	Aryl hydrocarbon receptor (AhR) antagonist; promotes HSC expansion and engraftment
Troglitazone	3114	Selective PPARγ agonist; antidiabetic agent; inhibits cell growth of hematopoietic cell lines

Organoids

Product Name	Catalog #	Primary Action
A 83-01	2939	Selective inhibitor of TGF-β RI, ALK4, and ALK7; 3D growth matrix component and additive for long-term organoid growth
CHIR 99021	4423	Highly selective GSK-3β inhibitor; commonly used in organoid generation
DAPT	2634	γ-secretase inhibitor; induces neuronal differentiation; 3D growth matrix component
endo-IWR 1	3532	Wnt-β-Catenin signaling inhibitor; axin stabilizer; component of neocortex differentiation media
Forskolin	1099	Adenylyl cyclase activator; used in liver organoid generation
Galunisertib	6956	ALK4 and ALK5 (TGF β RI) inhibitor; component of growth media for urothelial organoids
Heparin sodium salt	2812	Used in protocol to generate kidney organoids

Organoids

Product Name	Catalog #	Primary Action
IWP 2	3533	PORCN inhibitor; inhibits Wnt processing and secretion; component of heart organoid differentiation media
IWP 4	5214	Potent inhibitor of Wnt/β-Catenin signaling; component of heart organoid differentiation media
LDN 193189	6053	Potent and selective ALK2 and ALK3 inhibitor; component of brain organoid differentiation media
Nicotinamide	4106	PARP-1 inhibitor; commonly used as 3D growth matrix component
N-Acetylcysteine	7874	Used in organoid media; glutathione (GSH) precursor
PD 0325901	4192	Potent inhibitor of MEK1/2; base media component
Prostaglandin E_2	2296	Major endogenous prostanoid; 3D gowth matrix component used in liver and prostate organoid generation
Retinoic acid	0695	Endogenous retinoic acid receptor agonist; 3D growth matrix component used in organoid generation
SB 202190	1264	Potent, selective inhibitor of p38 MAPK; 3D growth matrix component used in organoid generation
SB 431542	1614	Potent, selective inhibitor of TGF-β RI, ALK4, and ALK7; 3D growth matrix component used in organoid generation
Tissue Clearing Pro-Organoid	7390	3D cell culture clearing reagent kit
Y-27632	1254	Selective ROCK inhibitor; 3D growth matrix component used in organoid generation

Reprogramming

Product Name	Catalog #	Primary Action
(+)-Bay K8644	1544	$Ca_v1.x$ activator; promotes generation of iPSCs from MEFs
5-BrdU	5015	Synthetic thymidine analog; replaces Oct-4 in transcription factor-mediated reprogramming
BIRB 796	5989	High affinity and selective p38 kinase inhibitor; used in protocols to generate iPSCs
CHIR 98014	6695	Highly potent and selective GSK-3β inhibitor; used in differentiation and reprogramming of stem cells
CHIR 99021	4423	Highly selective GSK-3β inhibitor; used in small molecule cocktail to generate ciPSCs from MEFs
DBZ	4489	γ-secretase inhibitor; inhibits Notch pathway; promotes formation of iPSCs
3-Deazaneplanocin A	4703	EZH2 histone methyltransferase inhibitor; widely used in chemical reprogramming protocols as it promotes expresson of Oct4 in iPSCs
(S)-(+)-Dimethindene maleate	1425	Selective ACh muscarinic M_2 antagonist; used in generation of extended pluripotent stem (EPS) cells
Kenpaullone	1398	GSK-3β inhibitor; also inhibits CDKs; promotes generation of iPSCs from somatic cells
Linifanib	7743	Potent receptor tyrosine kinase (RTK) inhibitor; promotes the generation and reprogramming of iPSCs from somatic cells
Minocycline hydrochloride	3268	Antibiotic; allows formation of extended pluripotent stem (EPS) cells
Neurodazine	3656	Induces neurogenesis in mature skeletal muscle cells
O412	5664	Oct3/4 inducer; induces expression of pluripotency-associated genes
PD 0325901	4192	Potent inhibitor of MEK1/2; enhances generation of iPSCs
RepSox	3742	Potent and selective inhibitor of TGF-β RI; enhances reprogramming efficiency
RG 108	3295	Non-nucleoside DNA methyltransferase inhibitor; enhances efficiency of iPSC generation

Reprogramming			
	Product Name	**Catalog #**	**Primary Action**
	Ruxolitinib	7064	Potent and selective JaK1/2 inhibitor; used in a protocol to reprogram HEFs into iPSCs
	SB 431542	1614	Potent, selective inhibitor of TGF-β RI, ALK4, and ALK7; replaces SOX2 in reprogramming of fibroblasts into iPSCs
	SB 590885	2650	Potent B-Raf inhibitor; used in a protocol to reprogram HEFs into iPSCs
	SGC-CBP30	4889	Potent CBP/p300 BRD inhibitor; used in protocols to generate iPSCs
	SMER 28	4297	Positive regulator of autophagy; promotes reprogramming of fibroblasts to neural stem-like cells
	Thiazovivin	3845	ROCK inhibitor; improves the efficiency of fibroblast reprogramming and induction of iPSCs
	Tranylcypromine hydrochloride	3852	Irreversible inhibitor of LSD1; also inhibits MAO; enables reprogramming of mouse embryonic fibroblasts into iPS cells
	Trichostatin A	1406	Potent histone deacetylase inhibitor; accelerates reprogramming of primordial germ cells to PSCs
	TTNPB	0761	Retinoic acid analog; RAR agonist; enhances efficiency of reprogramming in ciPSCs
	UNC 0224	3861	Potent G9a and GLP inhibitor; used in a protocol to reprogram HEFs into iPSCs
	Valproic acid, sodium salt	2815	Histone deacetylase inhibitor; enhances efficiency of reprogramming of somatic cells
Self-Renewal			
	A 769662	3336	Potent AMPK activator; inhibits MSC proliferation
	A 77-01	6712	Potent inhibitor of TGF-β RI; likely active metabolite of A 83-01 (Catalog # 2939)
	A 83-01	2939	Selective inhibitor of TGF-β RI, ALK4, and ALK7; maintains self-renewal of human iPSCs
	AS 1842856	4265	Potent and selective FOXO1 inhibitor; suppresses differentiation of adipocytes
	BIO	3194	Potent GSK-3β inhibitor; also inhibits CDKs; maintains self-renewal and pluripotency of ESCs
	CDK8/19i	7372	Potent and selective CDK8 and CDK19 inhibitor; maintains pluripotency of mouse PSCs in culture
	Chroman 1	7163	Highly potent and selective ROCK inhibitor; maintains survival of hPSCs
	INDY	4997	DYRK1A/B inhibitor; imparis self-renewal of NSCs
	Kyoto Probe-1	7419	Fluorescent probe that selectively identifies undifferentiated iPS/ES cells
	LY 294002 hydrochloride	1130	Prototypical PI 3-kinase inhibitor; also inhibits other kinases; suppresses proliferation of mESCs
	PD 173074	3044	FGRR1 and -3 inhibitor; inhibits proliferation and differentiation of oligodendrocyte progenitors
	PD 98059	1213	MEK inhibitor; enhances ESC self-renewal
	Pioglitazone hydrochloride	4124	Acts synergistically with Y-27632 (Catalog # 1254) to improve PSC cloning efficiency; PPARγ agonist
	EHNA hydrochloride	1261	Adenosine deaminase inhibitor; suppresses spontaneous differentiation of human ESCs
	Go 6983	2285	Broad spectrum PKC inhibitor; optimizes naïve human pluripotent stem cell growth and viability
	Emricasan	7310	Promotes survival of hPSCs in combination with other small molecules; potent pan-caspase inhibitor

Self-Renewal			
	Product Name	**Catalog #**	**Primary Action**
	Epiblastin A	6340	CK1 inhibitor; converts epiblast stem cells to ESCs and promotes ESC self-renewal
	FzM1.8	6961	Frizzled 4 allosteric agonist; exhibits biased signaling; preserves stemness
	Pyrintegrin	4978	Enhances survival of human ESCs following enzymatic dissociation
	SB 202190	1264	Potent, selective inhibitor of p38 MAPK; promotes stability of human PSCs
	SB 203580	1202	Selective inhibitor of p38 MAPK; stimulates neural stem cell proliferation
	SB 216763	1616	Potent, selective GSK-3β inhibitor; maintains pluripotency of mouse ESCs
	SP 600125	1496	Selective JNK inhibitor; used for maintaining stem cells in naïve pluripotent state
	SR 3677	3667	Potent, selective Rho-kinase (ROCK) inhibitor
	SU 5402	3300	Potent FGF R and VEGF R inhibitor; supports mESC self-renewal
	Surfen	6634	Heparin sulfate antagonist; maintains pluripotency of hESCs
	trans-ISRIB	5284	Promotes survival of hPSCs in combination with other small molecules; integrated stress response (ISR) inhibitor
	U0126	1144	Potent, selective inhibitor of MEK1 and 2; maintains hPSCs self-renewal
	WH-4-023	5413	Potent and selective Lck and Src inhibitor; also inhibits SIK; supports self-renewal of naïve hESCs
	Y-27632	1254	Selective ROCK inhibitor; increases survival of human embryonic stem cells undergoing cryopreservation
	Yhhu 3792	6599	Notch signaling pathway activator; enhances the self-renewal capability of NSCs
Stem Cell Signaling			
Hedgehog	Cyclopamine	1623	Inhibitor of Hedgehog (Hh) signaling; induces differentiation of hESCs into hormone expressing endocrine cells
	GANT 61	3191	GLI antagonist; inhibits Hedgehog (Hh) signaling
	Hh-Ag1.5	7807	Potent and high affinity Smo receptor agonist; induces differentiation of PSCs into spinal motor and sensory neurons
	20(S)-Hydroxycholesterol	4474	Allosteric activator of Hedgehog (Hh) signaling; induces Smo accumulation
Integrins	BIO 5192	5051	Highly potent and selective inhibitor of integrin α4β1 (VLA-4); mobilizes HSCs and progenitors
	BOP	6047	Dual α9β1/α4β1 integrin inhibitor; preferentially mobilizes HSCs
TGF-β/BMP	Dorsomorphin dihydrochloride	3093	BMP type I receptor inhibitor; also potent AMPK inhibitor promotes cardiomyocyte differentiation in mouse ESCs
	SB 4	6881	Potent BMP4 agonist
Wnt/β-Catenin	BIO	3194	Potent GSK-3β inhibitor; also inhibits CDKs; maintains self-renewal and pluripotency of ESCs
	endo-IWR 1	3532	Wnt/β-Catenin signaling inhibitor; axin stabilizer; promotes endothelial cell specification of cardiac progenitors
	IWP 2	3533	PORCN inhibitor; inhibits Wnt processing and secretion
	QS 11	3324	ARFGAP1 inhibitor; modulates Wnt/β-Catenin signaling
	WIKI4	4855	Potent tankyrase inhibitor
Other	17-AAG	1515	Selective HSP90 inhibitor; protects neuroprogenitor cells against stress-induced apoptosis
	Mitomycin C	3258	DNA cross-linking anti-tumor agent; used for MEF/STO feeder layer preparation in stem cell culture
	Pifithrin-α hydrobromide	1267	p53 inhibitor; also aryl hydrocarbon receptor agoinst; inhibits ESC self-renewal

Stem Cell Products in Solution			
	Product Name	**Catalog #**	**Primary Action**
	CHIR 99021 in solution	8170	Sterile-filtered 10 mM solution of CHIR 99021 pre-dissolved in DMSO
	LDN 193189 in solution	8150	Sterile-filtered 10 mM solution of LDN 193189 pre-dissolved in water
	Y-27632 in solution	7000	Sterile-filtered 10 mM solution of Y-27632 pre-dissolved in water
Other Stem Cell Products			
Compound Libraries	Tocriscreen Stem Cell Library	7340	A library of 120 stem cell compounds (100 µL, 10 mM DMSO solutions) to explore stem cell reprogramming, differentiation, proliferation, and signaling
	AZD 7762	5199	Enhanes CRISPR-Cpf1-mediated genome editing; also potent and selective ATP-competitive Chk1 and Chk2 inhibitor
	Brefeldin A	1231	Enhances CRISPR-mediated HDR efficiency
	(Z)-4-Hydroxytamoxifen	3412	Activates intein-linked inactive Cas9, reducing off-target CRISPR-mediated gene editing
	KU 0060648	4840	Enhances HDR efficiency and attenuates NHEJ frequency
	L-755, 507	2197	Enhances CRISPR-mediated HDR efficiency
	Nocodazole	1228	Enhances HDR efficiency; also increases Cas9-mediated gene editing frequencies
	NU 7441	3712	Enhances HDR efficiency and attenuates NHEJ frequency
	SCR7 pyrazine	5342	Enhances HDR efficiency
ExtraCellular Matrix	RGD peptide	7723	Potent integrin inhibitor; can be incorporated into hyaluronic acid hydrogel for hMSCs delivery and increases hMSC spreading
Fluorescent Probes	DC 271	6873	Fluorescent retinoic acid analog; solvochromatic probe
	Kyoto Probe-1	7419	Fluorescent probe that selectively identifies undifferentiated iPS/ES cells
Viral Transduction Enhancers	Akti-1/2	5773	Enhances CAR and TCR retroviral transduction of human T cells; potent and selective dual Akt1 and 2 inhibitor
	Cyclosporin A	1101	Enhances lentiviral transduction; calcineurin inhibitor
	Cyclosporin H	6982	Enhances lentiviral transduction
	Daunorubicin hydrochloride	1467	Enhances adenoviral transduction; RNA synthesis inhibitor
	16,16-Dimethyl Prostaglandin E_2	4027	Enhances lentiviral transduction; synthetic prostaglandin E_2 (Catalog # 2296) derivative
	Etoposide	1226	Enhances adenoviral transduction; topoisomerase II inhibitor
	Polybrene	7711	Enhances lentiviral and adenoviral transduction efficiency; also enhances DNA transfection in various cell types
	Prostaglandin E_2	2296	Enhances lentiviral transduction; endogenous prostanoid
	Rapamycin	1292	Enhances lentiviral transduction; mTOR inhibitor and immunosuppressant
	SAHA	4652	Enhances plasmid transduction; class I and II HDAC inhibitor
	Staurosporine	1285	Enhances lentiviral transduction; non-selective protein kinase inhibitor
	Teniposide	6975	Enhances adenoviral transduction; topoisomerase II inhibitor

A Selection of Related Products Available from R&D Systems

Select Recombinant Proteins for Stem Cell and Organoid Culture		
Molecule	**Species**	**Catalog #**
Activin A	Human	11348-AC
Activin A Hyperactive	Human	BT-ACTAH
BDNF	Human	BT-BDNF
Betacellulin	Human	BT-BTC
BMP-4	Human	314-BPE
EGF	Human	236-EG
FGF-basic	Human	BT-FGFB
FGF-basic Heat Stable	Human	BT-FGFBHS
FGF-4	Human	7460-F4
FGF-8b	Human/Mouse	423-F8
FGF-9	Human	273-F9
GDNF	Human	212-GD
HGF	Human	294-HGN
IGF-I/IGF-1	Human	291-G1
KGF/FGF-7	Human	BT-KGF
LIF	Human	7734-LF
Noggin	Human	6057-NG
PDGF-AA	Human	221-AA
PDGF-BB	Human	220-BB
Sonic Hedgehog/Shh	Human	1845-SH
TGF-beta 1	Human	7754-BH
TGF-beta 2	Human	302-B2
TGF-beta 3	Human	8420-B3
Wnt-3a	Human	5036-WN
Wnt-5a	Human	645-WN
Wnt/RSPO1 Agonist	Human	BT-WRSP1
Wnt-RSPO2 Agonist	Human	BT-WRSP2
Wnt-RSPO3 Agonist	Human	BT-WRSP3
Select Animal-Free Preclinical Proteins		
Activin A	Human/Mouse/Rat	AFL338
BDNF	Human	BT-BDNF-AFL
Betacellulin	Human	BT-BTC-AFL
BMP-4	Human	AFL314E
EGF	Human	AFL236
FGF basic	Human	BT-FGFB-AFL
FGF basic Heat Stable	Human	BT-FGFBHS-AFL
IGF-I/IGF-1	Human	AFL291
KGF/FGF-7	Human	BT-KGF-AFL

Select Animal-Free Preclinical Proteins		
Molecule	**Species**	**Catalog #**
PDGF-AA	Human	AFL221
PDGF-BB	Human	AFL220
Sonic Hedgehog	Human	AFL1845
VEGF	Human	BT-VEGF-AFL
Select GMP-grade Proteins		
Activin A	Human/Mouse/Rat	338-GMP*
BDNF	Human	BT-BDNF-GMP*
Betacellulin	Human	BT-BTC-GMP*
BMP-2	Human	355-GMP
BMP-4	Human	314E-GMP
EGF	Human	236-GMP*
FGF-basic	Human	BT-FGFB-GMP*
FGF-basic Heat Stable	Human	BT-FGFBHS-GMP
GDNF	Human	212-GMP
HGF	Human	294-GMP
IGF-I/IGF-1	Human	291-GMP*
KGF/FGF-7	Human	BT-KGF-GMP
Noggin	Human	3344-GMP
PDGF-AA	Human	221-GMP
PDGF-BB	Human	220-GMP
Sonic Hedgehog/Shh	Human	1845-GMP
TGF-beta 1	Human	240-GMP*
VEGF	Human	BT-VEGF-GMP*
Wnt-3a	Human	5036-GMP
* DMF have been filed for these GMP proteins.		

Select Antibodies for Stem Cell Identification		
Molecule	**Species**	**Unconjugated Ab Catalog #**
5' Nucleotidase/CD73	Human	MAB5795
	Mouse	MAB4488
CD34	Human	MAB7277
	Mouse	MAB6518
CD43	Human	MAB2038
CD44	Human/Mouse	MAB6127
CD48	Human	MAB3644
	Mouse	MAB3327

Select Antibodies for Stem Cell Identification		
Molecule	**Species**	**Unconjugated Ab Catalog #**
CD90/Thy1	Human	MAB2067
	Mouse	MAB7335
CD105/Endoglin	Human	MAB10791
	Mouse	MAB1320
CD117/c-kit	Human	MAB332
	Mouse	MAB1356
CD133	Human	MAB11331
CD150/SLAM	Human	MAB1642
	Mouse	MAB4330
Musashi-1	Human/Mouse/Rat	AF2628
Nanog	Human	AF1997
Nestin	Human	MAB1259
	Mouse/Rat	MAB2736
Oct-3/4	Human/Mouse	MAB1759
Pax6	Human	AF8150
Sca-1 (mouse)	Mouse	MAB1226
SOX2	Human/Mouse	MAB2018
SSEA-1 (mouse)	Human/Mouse	MAB2155
SSEA-3	Human/Mouse	MAB1434
SSEA-4 (human)	Human/Mouse	MAB1435
TRA-1-60(R)	Human	MAB4770

Select Stem Cell Media and Supplements	
Product Name	**Catalog #**
Cultrex™ Stem Cell Qualified RGF Basement Membrane Extract	3434-010-02
Cultrex RGF Basement Membrane Extract, Type 2	3533-010-02
Cultrex UltiMatrix RGF Basement Membrane Extract	BME001-05
ExCellerate™ iPSC Expansion Medium, Animal Component-Free	CCM036
ExCellerate iPSC Expansion Medium, Animal-Free, GMP	CCM036-GMP
N-2 MAX Media Supplement	AR009
N21-MAX Media Supplement	AR008
StemXVivo™ Chondrogenic Base Media	CCM005
Chondrogenic Supplement	CCM006
StemXVivo Osteogenic/Adipogenic Base Media	CCM007
Adipogenic Supplement	CCM011
Human Osteogenic Supplement	CCM008
Mouse/Rat Osteogenic Supplement	CCM009
Stem Cell Differentiation Kits	
StemXVivo Ectoderm Kit	SC031B
StemXVivo Endoderm Kit	SC019B
StemXVivo Hepatocyte Differentiation Kit	SC033
StemXVivo Mesoderm Kit	SC030B
StemXVivo Neural Progenitor Differentiation Kit	SC035
Stem Cell Functional Identification Kits	
Human Mesenchymal Stem Cell Functional Identification Kit	SC006
Mouse Mesenchymal Stem Cell Functional Identification Kit	SC010
Human Pluripotent Stem Cell Functional Identification Kit	SC027B

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SELF-RENEWAL/ MAINTENANCE

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