InvivoGen Insight

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Inside this issue:

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Mycoplasma: the insidious enemy

It's the forbidden word in cell culture. It is every cell culturist's nightmare, or at least it should be. Mycoplasma. Cell culture contaminations of mycoplasma have been known for decades. The problem is serious, and with potentially 50% or more of cell-lines worldwide contaminated, mycoplasma can significantly impact the interpretation of biological results. To what extent and measures should we go to prevent or eliminate Mycoplasma contamination in cell culture?

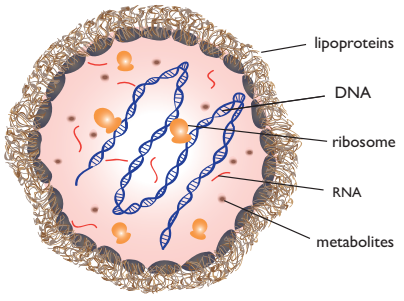
Mycoplasma is a unique type of microorganism belonging to the class of *Mollicutes*. The first strains of mycoplasma were isolated at the Pasteur Institute in 1898, and to date 20 of the 190 or so species have been identified as *bona fide* contaminants in laboratory cell culture. Mycoplasmas are the smallest free-living organisms and considered the simplest of bacteria. Owing to their extremely basic genomes, mycoplasmas in fact are parasites exploiting host cells to fulfill their energy requirements and biosynthesis of their components.

Since first reported in the mid-1950s, mycoplasma contamination of culture cell lines has become a major problem in both basic research and in the production of biopharmaceuticals[1,2]. Mycoplasmas are structurally unlike other bacteria in that they lack a solid wall, instead possessing a "plasma"-like form. This, together with their minute size (~100 nm), means that mycoplasma contaminations go undetected for extended lengths of time and are resistant to a number of commonly used antibiotics, such as penicillin and streptomycin. Hundreds of mycoplasmas can attach to a single eukaryotic cell, eventually invading the host by fusing with the cell membrane. Upon entry into the cell, mycoplasmas multiply and circumvent defenses to survive, outnumbering host cells by 1000-fold. Contamination of a cell culture by mycoplasmas cannot be visualized by the naked eye or even by light microscopy and do not produce overt turbid growth commonly associated with bacterial and fungal contamination. The morphological cellular changes and growth rates of cell cultures can be minimal or unapparent.

Given the high and potentially increasing incidence of mycoplasma contamination in cell lines worldwide, the issue cannot be ignored by any laboratory conducting cell culture as the manifestations are profound. Mycoplasma competes with host cells for biosynthetic precursors and nutrients and can alter DNA, RNA and protein synthesis, diminish amino acid and ATP levels, introduce chromosomal alterations and modify host-cell plasma membrane antigens. A microarray analysis on contaminated cultured human cells revealed the extortionate effects that mycoplasma has on altering the expression of hundreds of genes including those encoding for receptors, ion channels, growth-factors, and oncogenes[3]. In addition, mycoplasmas exert significant effects on cultured immune cells such as monocytes and macrophages. Mycoplasmas contain highly immunogenic lipoproteins anchored on the outer face of the plasma membrane. These lipoproteins are recognized by specific pattern recognition receptors, in particular Toll-like receptor 2 (TLR2) present on immune cells. Upon recognition of the mycoplasmal lipoproteins, TLR2 induces the NF-κB pathway leading to the activation of these cells therefore biasing experimental results.

There are three major sources leading to mycoplasma contamination of cell cultures: 1) incoming infected cells, 2) contaminated cell culture medium ingredients such as serum and trypsin 3) laboratory personnel infected with *M. orale or M. fermentans*. A contamination can spread rapidly to other cell lines through aerosol droplet dispersion. Once mycoplasmas have been detected, discarding the contaminated cell line remains the best-recommended solution to eliminate and prevent spreading. In important cases, for instance to rescue a valuable cell line, an effective mycoplasma eradication treatment is needed. Some antibiotics that are selectively active on mycoplasma are currently available. Following treatment, they have been shown to eliminate mycoplasma and restore cell behavior and responses[5].

So the answer is clear and simple. All efforts must be taken to prevent or eliminate mycoplasma contamination in cell culture. Regular testing of cell cultures to ensure absence of mycoplasma in conjunction with good aseptic technique is strongly advised. InvivoGen offers an easy and reliable mycoplasma detection method, PlasmoTest™. This cell-based assay exploits the ability of the immune system, in particular TLR2, to recognize mycoplasma and can be easily implemented as part of cell culture to routinely check for all types of mycoplasma contamination. Cell cultures found contaminated can be effectively treated with Plasmocin™, a well-established antimycoplasma reagent, or Plasmocure™, an alternative mycoplasma removal agent, some of InvivoGen's best sellers.



1. Robinson LB. *et al.*, 1956. Contamination of human cell cultures by pleuropneumonia like organisms. Science. 124(3232):1147-8. **2. Armstrong SE. *et al.*, 2010.** The scope of mycoplasma contamination within the biopharmaceutical industry. Biologicals. 38(2):211-3. **3. Miller CJ. *et al.*, 2003.** Mycoplasma infection significantly alters microarray gene expression profiles. Biotechniques. 35(4):812-4. **4. Takeuchi O. *et al.*, 2001.** Discrimination of bacterial lipoproteins by Toll-like receptor 6. Int Immunol. 13(7):933-40. **5. Zakharova E. *et al.*, 2010.** Mycoplasma suppression of THP-1 Cell TLR responses is corrected with antibiotics. PLoS One. 5(3):e9900.

Mycoplasma Detection & Elimination

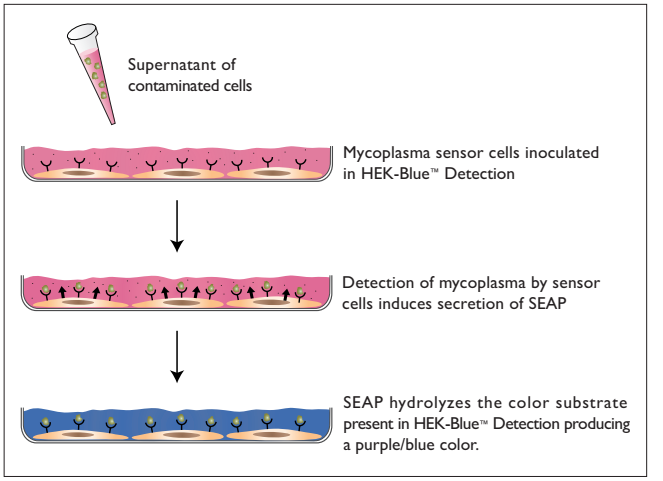
➢ PlasmoTest™

Mycoplasma detection right in your incubator

- **Simple -** Requires only basic cell culture knowledge. No need for specific lab equipment. Results are easily determined with the naked eye or quantified with a spectrophotometer.
- **Rapid -** Hands-on time less than 1 hour. Gives results after overnight incubation.
- **Versatile -** Detects all *Mycoplasma* and *Acholeplasma* species known to infect cell cultures, as well as other cell culture contaminants such as bacteria.
- **Sensitive -** Detects 5.10^2-5.10^5 cfu/ml mycoplasmas. No false positive: a positive result indicates the presence of a cell culture contaminant.
- **Low Cost per Test -** Designed for frequent mycoplasma testing.
- **Complete -** Contains the Mycoplasma sensor cells and all the reagents needed to perform the assay, including positive and negative controls. Up to 500 samples can be tested with the kit. To perform further assays, only the reagents need to be reordered.

InvivoGen's PlasmoTest™ is the first assay to utilize cells to signal the presence of mycoplasma. It exploits the ability of the innate immune system, in particular Toll-like receptor 2 (TLR2), to recognize mycoplasmas. TLR2 is a pathogen-associated molecular pattern recognition receptor that recognizes distinct lipoproteins anchored on the plasma membrane of mycoplasmas. Upon recognition, TLR2 induces a signaling cascade leading to the activation of the transcription factor NF-κB.
PlasmoTest™ consists of engineered HEK293 cells, the Mycoplasma Sensor Cells, that express TLR2 and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. Therefore, when the Mycoplasma Sensor cells detect mycoplasma through TLR2, they produce SEAP which is detected using a simple colorimetric assay, HEK-Blue™ Detection.

PlasmoTest™ Principle



Buy PlasmoTest™ once and then simply reorder the reagents, HEK-Blue™ Detection and PlasmoTest™ Controls, to perform further assays.

PRODUCT	QUANTITY	CAT. CODE
PlasmoTest™	1 kit	rep-pt2
HEK-Blue™ Detection	2 pouches	hb-det1
PlasmoTest™ Controls	200 tests	pt-ctr2

➢ Plasmocin™

Eradicate mycoplasma in cell cultures and primary cells

- **Effective -** Kills all common mycoplasma strains, both free and intracellular forms.
- **Fast -** Rescues cell cultures in 2 weeks.
- **Safe -** No to little cytotoxic effects on mammalian cells.
- **Simple -** Just add to cell culture medium at the recommended concentration.
- **Preventive -** At lower concentration, prevents re-infection.

Over the years, InvivoGen's Plasmocin™ has proven to be a lifesaver for cultures of thousand of researchers. It contains two bactericidal components strongly active against mycoplasmas allowing their elimination in only 2 weeks. These components act on two specific and separate targets found solely in mycoplasmas. This ensures that there is no irreversible toxic side effects that could damage your cells.

Many cell lines infected by mycoplasmas have been successfully treated with Plasmocin™, including murine embryonic stem cells, hybridomas, lymphocytes, epithelial cells and retrovirus packaging cells.

Two formulations of Plasmocin™ are offered:
- Plasmocin™ Treatment

To eliminate mycoplasmas use Plasmocin™ Treatment at 25 µg/ml for two weeks in the infected cell culture.
- Plasmocin™ Prophylactic

To prevent mycoplasma contamination use Plasmocin™ Prophylactic 5 µg/ml on a regular basis in cell cultures.

Six species of *Mycoplasma* and related cell wall-less bacteria account for 95% of all *Mollicutes* contaminants in cell cultures: *Mycoplasma (M.) arginini, M. fermentans, M. orale, M. hyorhinis, M. salivarum* and *Acholeplasma laidlawii*. Plasmocin™ has proven to be effective against all 6 species.

➢ Plasmocure™

In very rare cases, mycoplasmas resistant to Plasmocin™ have been reported. To eradicate these mycoplasmas, InvivoGen has developed a new antimycoplasma agent called Plasmocure™. Plasmocure™ combines two antibiotics that act through different mechanisms of action than those in Plasmocin™. A two week treatment with Plasmocure™ was found sufficient to completely eliminate the mycoplasmas.

PRODUCT	QUANTITY	CAT. CODE
Plasmocin™ Treatment	50 mg (2 x 1 ml)	ant-mpt
Plasmocin™ Prophylactic	25 mg (5 x 2 ml)	ant-mpp
Plasmocure™	100 mg (1 ml)	ant-pc

Inflammasome

The inflammasome is a multiprotein complex that activates caspase 1, leading to the processing and secretion of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18. Each inflammasome includes a member of the nucleotide oligomerization domain-like receptor (NLR) family of proteins. Diverse pathogen-associated molecular patterns (PAMPs) and non-microbial danger-associated molecular patterns (DAMPs) are sensed intracellularly by NLRs. The subsequent oligomerization of NLRs results in NLR interaction with ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), a central adaptor protein of inflammasome. ASC then interacts with pro-caspase-1 yielding cleavage and activation of caspase-1, which leads to the maturation of pro-IL-1β to active IL-1β. A number of inflammasomes have been described, including the NLRP1, NLRP3 and NLRC4 inflammasomes. The NLRP3 inflammasome is the most extensively studied and also the most versatile due to its broad recognition of a wide range of PAMPs and DAMPs. It is however still unclear how these highly diverse signals can be detected by a single inflammasome. To foster your research on the inflammasome, InvivoGen has developed a variety of cell-based tools.

Inflammasome Test Cells

THP-1 human monocytic cells represent the most commonly used model cell line for the study of inflammasome activation as they express high levels of NLRP3, ASC and pro-caspase-1. InvivoGen provides three engineered THP-1 cell lines as tools to determine whether a signal activates the inflammasome and in particular the NLRP3 inflammasome.

➢ THP1-defASC cells NEW

THP1-defASC cells express negligible levels of ASC but express native levels of NLRP3 and pro-caspase-1. These cells are unable to respond to inducers of ASC-dependent inflammasomes, such as inducers of the NLRP3 inflammasome.
Application: To determine if a given signal is an inflammasome inducer.

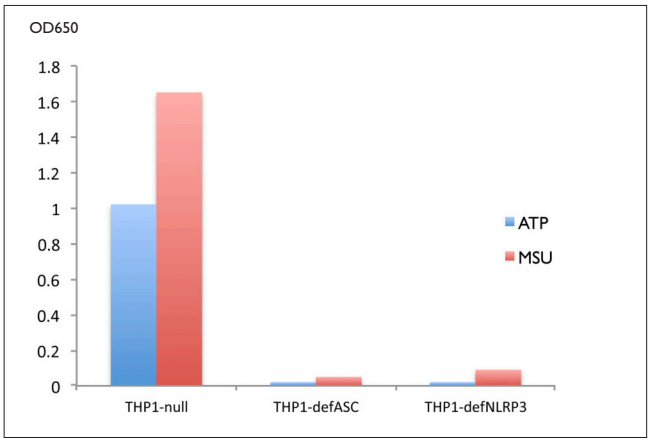
➢ THP1-defNLRP3 cells NEW

THP1-defNLRP3 cells are deficient for NLRP3 activity but proficient for ASC and caspase-1 activities. Thus, these cells are unable to respond to inducers of the NLRP3 inflammasome, such as ATP and MSU (see figure). However, they may respond to signals that activate other ASC-dependent inflammasomes such as NLRP1 and NLRC4 inflammasomes.
Application: To study the involvement of NRLP3 in response to a given signal.

➢ THP1-Null cells NEW

THP1-Null cells are fully efficient for NLRP3 and ASC activities. They produce IL-1β upon stimulation with inflammasome inducers.
Application: Use as positive control cell line for inflammasome studies.

All three cells lines are resistant to hygromycin.



IL-1β production in THP1-null, THP1-defASC and THP1-defNLRP3 cells following their stimulation with ATP and MSU. Cells primed with LPS (1 µg/ml) were stimulated with ATP (5 mM) or MSU (100 µg/ml). After 24h incubation, the supernatants were added to HEK-Blue™ IL-1β cells. IL-1β-induced activation of NF-κB was assessed by measuring the levels of SEAP in the supernatant of HEK-Blue™ IL-1β cells using the QUANTI-Blue™ assay.

IL-1β Reporter Cells

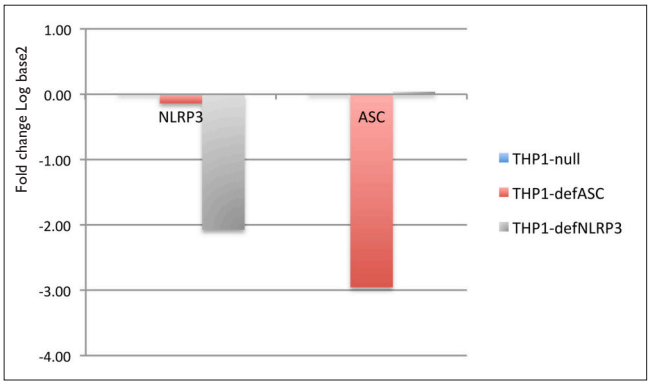
HEK-Blue™ IL-1β provides a convenient read-out to monitor the production of IL-1β from THP1 cells following their stimulation by inflammasome inducers.

➢ HEK-Blue™ IL-1β cells

HEK-Blue™ IL-1β cells are HEK293 cells that stably express an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. They naturally express the IL-1 receptor. Upon IL-1β binding to its receptor, a signaling cascade is initiated triggering NF-κB activation and the subsequent production of SEAP. Levels of SEAP can be readily assessed using QUANTI-Blue™, a SEAP detection medium.

HEK-Blue™ IL-1β cells are resistant to Zeocin™ and hygromycin.

InvivoGen also provides HEK-Blue™ IL-18/IL-1β cells for the detection of bioactive IL-18 and IL-1β. HEK-Blue™ IL-18/IL-1β cells are derived from HEK-Blue™ IL-1β cells by stable transfection of the IL-18RAP gene. For more information, go to http://www.invivogen.com/hek-blue-il18-il1b.



Quantitative RT-PCR analysis showing the fold change of NLRP3 and ASC genes in THP1-defASC and THP1-defNLRP3 cells compared to THP1-null cells.

PRODUCT	QTY	CAT. CODE
THP1-Null	5-7 x 10^6 cells	thp-null
THP1-defASC	5-7 x 10^6 cells	thp-dasc
THP1-defNLRP3	5-7 x 10^6 cells	thp-dnlp
HEK-Blue™ IL-1β	5-7 x 10^6 cells	hkb-il1b

Immunomodulatory Compound Screening

TLR, NOD and RLR Ligand Screening Service

There is a growing interest in the targeting of Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) for drug discovery research. As a recognized industry leader in innate immunity, InvivoGen provides a high quality immunomodulatory compound screening service to assist our clients' drug discovery and development needs.

- **Short turnaround time -** Screening turnaround: ONLY 3 weeks
- **Screening flexibility -** Screening parameters can be selected and/or modified based on customer requirements.
- **Cost effective -** A set-up charge applies for the first compound. Subsequent compounds are heavily discounted.
- **Reliable -** Our screening service has been utilized consistently by leading Biotech and Pharmaceutical companies and academic institutes for many years.

Over the past several years InvivoGen has developed a large collection of cellular assays to detect compounds that activate or block the immune system through activation of PRRs with an emphasis on TLRs, NOD1/2 and RIG-I/MDA-5. These sensitive cellular assays feature an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) or an IRF (interferon regulatory factor)-inducible sLUC (secreted luciferase) reporter gene as the read-out. Upon stimulation, activation of the NF-κB or IRF pathways is monitored using proprietary detection assays designed to provide rapid and reliable results.

➤ TLR Ligand Screening

The TLR ligand screening service utilizes the HEK-Blue TLR cells. These HEK293-derived cells are NF-κB-SEAP reporter cells that stably express a human or mouse TLR gene. The TLR genes expressed are TLR2, 3, 4, 5, 7, 8, and 9. For more information, go to http://www.invivogen.com/hek-blue-tlr.

Compound profiling: The compound is tested on all human and/or mouse TLRs to determine which TLR recognizes this compound. TLR ligands are typically recognized by a single TLR or potentially two (TLR7 and TLR8). Recognition by TLR4 usually reflects the presence of endotoxins in the sample.

Agonist and antagonist assays can be performed.

➤ NOD1/2 Ligand Screening

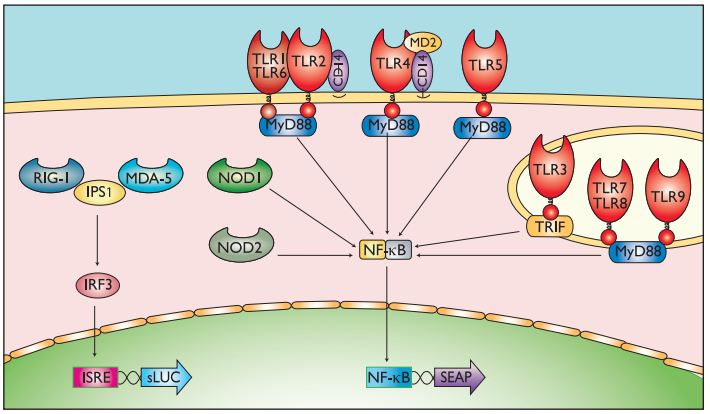
The NOD1/2 ligand screening service utilizes the HEK-Blue NOD cells. These HEK-Blue cells stably express an NF-κB-inducible SEAP reporter gene and the human or mouse NOD1 or NOD2 gene. For more information, go to http://www.invivogen.com/hek-blue-nod.

Compound profiling: The compound is tested on human and/or mouse NOD1 and NOD2.

➤ RIG-I/MDA-5 Ligand Screening NEW

InvivoGen introduces the RIG-I/MDA-5 ligand screening service. This service utilizes recently developed HEK293 cell lines that exploit the IRF pathway and a secreted luciferase reporter assay. They overexpress the human RIG-I or MDA-5 gene and are highly sensitive to 5'ppp-dsRNA and transfected poly(I:C), respectively, unlike their parental cell line that expresses low levels of both RIG-I and MDA-5.

Compound profiling: The compound is tested on human RIG-I and/or MDA-5.



Simplified representation of the TLR, NOD and RLR pathways

Screening Services

Two choices of services are offered, Compound Profiling and Compound Dose Response, that can be performed sequentially or individually.

- **Compound Profiling (level 1):** Single dose testing on a set of PRRs.

Screening is performed at a single concentration, typically a 1/10 dilution of the original compound solution provided, or customer specified.

- **Compound Dose Response (level 2):** Dose response on one or several PRRs

Three concentrations of the compound(s), typically 1/10, 1/100 and 1/1000 dilutions of the original compound solution, are tested on the PRR(s) recognizing the compound(s) as determined in level 1 or specified by the customer.

A detailed report is prepared and provided to the customer electronically and in hard copy. All procedures are performed accordingly to strict guidelines. Confidentiality is guaranteed.

PRODUCT	CAT. CODE
Compound Profiling	tlrl-test1
Compound Dose Response	tlrl-test2

Contact us for more information at info@invivogen.com

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