Cell Recovery Procedure

For InvivoGen's engineered HepG2 cells provided in shipping flask
http://www.invivogen.com/hepg2-dual

For research use only
Version 18E29-MM

HANDLING PROCEDURES

Contents and Storage

- 1 flask of engineered HepG2 cells in growth medium
- 2 x 20 ml syringes
- 1 x 5 ml syringe
- 4 blunt-end needles

Cell Handling Upon Arrival

- Inspect the package in order to detect any leak. If a leak is detected, contact InvivoGen immediately.
- Check the cells using a microscope.
- Process **as soon as possible** upon receipt.

Note: If the cells cannot be handled immediately, place the flask at room temperature (15-25°C) for a maximum of 48 hours in a horizontal position.

- DO NOT STORE AT 4°C**.**
- **Transfer flask to an incubator at 37°C in 5% CO_2.**
- Incubate for 12-24 hours in a **HORIZONTAL** position.

Required Cell Culture Media

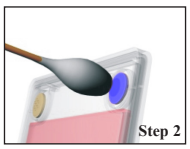
- Growth Medium: Eagle's minimal essential medium (EMEM), 10% (v/v) heat-inactivated fetal bovine serum (FBS; 30 min at 56°C), 1X non-essential amino acids (NEAA), Pen-Strep (100 U/ml-100 µg/ml), 100 µg/ml Normocin™
- Freezing Medium: EMEM with 20% (v/v) FBS and 10% (v/v) DMSO

Cell Recovery

HepG2 cells are adherent cells, however, during shipping the cells may detach from the surface of flask.

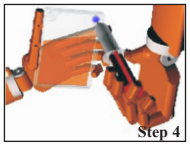
The shipping flask should always be manipulated in a VERTICAL position.

1. Following a 12-24 hr incubation in a HORIZONTAL position at 37°C in 5% CO_2, recover cells according to the procedure described below.
2. Disinfect blue port with 70% ethanol.
3. Shake gently the shipping flask for 10-15 seconds to detach the cells.



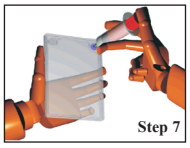
4. Whilst holding the shipping flask in a vertical position, withdraw SLOWLY all the medium (~20 ml) through the blue port using a 20 ml syringe with needle (provided).

Note: If the blue port is blocked, see Troubleshooting.



5. Transfer the cells to a 50 ml sterile conical tube.
6. Check cell recovery under the microscope. If all the cells have been recovered proceed to step 13.
7. Inject 3 ml of 0.25% trypsin/EDTA solution through the blue port using the 5 ml syringe with needle.
8. Shake thoroughly and incubate at 37°C for 5-10 minutes in a horizontal position.

*Note: Do **not** expose cells to trypsin for more than 10 minutes.*



9. Tap the shipping flask 2-3 times to detach the cells.
10. Inject 10 ml of fresh growth medium through the blue port using a new 20 ml syringe with needle.
11. Shake gently the shipping flask WITHOUT removing the syringe.
12. Withdraw SLOWLY the medium and add to the conical tube containing the previously collected 20 ml of medium.
13. Centrifuge the vial at 1000-1200 RPM (RCF 200-300 g) for 5 minutes.
14. Remove supernatant and resuspend cells with 1 ml of growth medium without selective antibiotics.
15. Separate the cells by pipetting up and down.
16. Transfer the cells to a T-75 culture flask containing 14 ml of growth medium without selective antibiotics.
17. Incubate at 37°C in 5% CO_2 until adherence.

Note: It may take 7 days for the cells to fully recover.

18. Assess cell viability and proceed to cell expansion or frozen stock preparation.

Frozen Stock Preparation

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Resuspend cells at a density of 3-5 x 10^6 cells/ml in freezing medium freshly prepared with cold growth medium.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Dispense 1 ml of cell suspension into cryogenic vials.
3. Place vials in a freezing container and store at -80ºC overnight.
4. Transfer vials to liquid nitrogen for long term storage.

Notes:

- If properly stored, cells should remain stable for years.
- Recover thawed cells in growth medium without selective antibiotics.

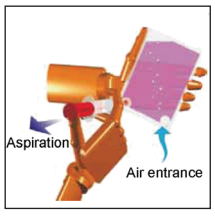
Cell Maintenance

1. Maintain and subculture the cells in growth medium supplemented with the appropriate selective antibiotic(s) (see original cell line datasheet).
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Troubleshooting

Flow deblocking procedure (for shipping flask).

If media accidentally enters into the microfluid trap and reaches the filter, the inflow will be blocked.



To remedy this:

1. Place the shipping flask upside down. Tilt the shipping flask so that the air pocket is in the corner above the blue port.
2. Withdraw 7-10 ml of fluid (air or media) from the blue port. After a couple of seconds, air bubbles will appear emerging from the air filter. This indicates that the filter and microfluidic trap are liquid-free, and the normal flow has been re-established.
3. Continue the filling operation in the upright position.

Note: This operation can be repeated any time the port is blocked.

TECHNICAL SUPPORT (InvivoGen Asia)
Unit 106; 1F, 15W Phase 3 Hong Kong Science Park,
Pak Shek Kok, Hong Kong
Toll-free: +852 3622-3480
Fax: +852-3622-3483
E-mail: info.hk@invivogen.com