Signosis
Innovative Plate Assay Solutions

HepG2 cell line

Catalog Number: PC-005 (For Research Use Only)

Materials provided

One vial of 2 x 10^6 cells in Freezing Media. **IMPORTANT**: store the frozen cells in liquid nitrogen until you are ready to thaw and propagate them.

Handling cells upon arrival

It is strongly recommended that you propagate the cells by following instructions as soon as possible upon arrival.

Required Cell Culture Media

- **Complete Growth Media**
  In 500mL of DMEM, add 50mL FBS (10% final) and 5mL Penicillin/Streptomycin (1% final).

- **2x Freezing Media**
  Add 10% DMSO (final) to Complete Growth Media and sterile filter. Make fresh each time.

Materials required but not provided

(Can be substituted with a comparable third-party product)

- Dulbecco's Modified Eagle's Medium (DMEM) -- *Hyclone P/N SH30243.01*
- Fetal Bovine Serum (FBS) -- *Fisherbrand P/N 03-600-511*
- Penicillin/Streptomycin -- *Hyclone P/N SV30010*
- Trypsin – *Hyclone P/N SH30236.02*
- Phosphate-buffered saline (PBS) -- *Cellgro P/N 21-040-CV*
- DMSO -- *Sigma P/N D8418*

Initial Culture Procedure

1. Quickly thaw cells in a 37°C water bath with careful agitation. Remove from the bath as soon as the vial is thawed.
2. Transfer cells to a 100mm^2 dish (or T25cm^2 flask) containing 10-12ml of **Complete Growth Media.**
3. Gently rock the flask to ensure the cells are mixed well in the media. DO NOT PIPET.
4. Place the flask with cells in a humidified incubator at 37°C with 5% CO_2.
5. After cells adhere (wait at least 4 hours to overnight), **replace media** with fresh Complete Growth Medium.

Subculture Procedure

1. Subculture/passage cells when the density reaches 90-100% confluency.
2. Carefully remove the culture media from cells by aspiration.
3. Rinse cells with PBS, being careful to not dislodge attached cells. Then remove PBS by aspiration.
4. Add 1-2 mL trypsin/Tris-EDTA solution. DO NOT add directly to cells.
5. Incubate with trypsin for 2-5 minutes (or until detached). Confirm detachment by observation under the microscope.
6. Add 5-10ml of pre-warmed Complete Growth Media and gently pipet up and down to break the clumps.
7. Passage cells in 1:3 to 1:5 ratio when they reach 90% confluency.

Preparing frozen stocks

This procedure is designed for 100mm^{2}dish or T75cm^2 flask. Scale volumes accordingly to other vessels.

1. When cells reach 90-100% confluency, freeze down cells.
2. Detach cells according to "Subculture Procedure."
3. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 250 x g (or 2,000 RPM) for 5 minutes to collect the cells into a pellet.
4. Carefully aspirate the media and resuspend cells in 0.5mL complete growth media.
5. Add 0.5mL of 2X freezing media and gently resuspend by pipetting up and down.
6. Transfer 1mL of cells into a cryogenic vial.
7. Place cryogenic vial in a freezing container (*Nalgene # 5100-0001*) and store at -80°C freezer overnight.
8. Transfer cells to liquid nitrogen for long term storage.