

# SOP: Propagation of HepG2 (ATCC HB-8065)

ENCODE4 - Version 1

## Information

Name: HepG2

ATCC #: HB-8065

Organism: *Homo sapiens*, Human

Tissue: liver

Morphology: epithelial

Culture Properties: adherent

Biosafety Level: 1

Disease: hepatocellular carcinoma; 15-year-old adolescent Caucasian male

Application: This cell line is a suitable transfection host

Population Doubling Time: about 48 hours

## Material List

1. DMEM (CORNING Cellgro; Cat # 10-013-CV or 10-013-CM)
2. Heat Inactivated Fetal Bovine Serum (CORNING; Cat # 35-016-CV)
3. Penicillin-Streptomycin 10,000U (Life Technologies, Cat # 15140 or Corning Cellgro, Cat # 300-002-CI)
4. Phosphate Buffered Saline (1X PBS) w/o  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  (CORNING Cellgro; Cat # 21-040-CM)
5. TrypLE Express (Life Technologies; Cat # 12604)
6. T75, T182 tissue culture treated flasks
7. Graduated pipets (2, 5, 10, 25, 50 ml)
8. Freezing medium (95% growth medium containing 5% DMSO)
9. DMSO (Fisher; Cat # BP-231-100)
10. Cryovials (Sarstedt; Cat #72-694-006)
11. TC20 cell counter (Bio-Rad)
12. Counting Slides (Bio-Rad; Cat # 145-0011)
13. 0.40% Trypan Blue Dye (Bio-Rad; Cat # 145-0013)
14. Microscope

## **Growth Medium for HepG2**

DMEM

10% FBS

Pen-Strep (1X, 100U)

### **Procedure**

#### **A. Receipt of Frozen Cells and Starting Cell Culture**

- 1) Immediately place frozen cells in liquid nitrogen freezer storage until ready to culture.
- 2) When ready to start cell culture, quickly thaw cryovial in a 37 °C water bath.
- 3) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, then dispense contents of ampoule into a T75 flask with 20-25 ml of warm growth media.
- 4) Allow cells to recover overnight in 37°C, 5% CO<sub>2</sub> humidified incubator.
- 5) The next morning, the diluted DMSO-containing shipping/cryopreservation medium is aspirated from the cell layer and replaced with fresh medium.

#### **B. Sub-culture**

Volumes used in this protocol are for 75 cm<sup>2</sup> and/or 182 cm<sup>2</sup> flask; proportionally reduce or increase amount of dissociation medium needed for culture vessels of the other size.

- 1) Propagate cells until density reaches 70-80% confluence.
- 2) Remove and discard culture medium.
- 3) Briefly rinse cell layer with room temperature (or warm) 1X PBS. (To remove all trace of serum that contains trypsin inhibitor.)
- 4) Add 3 ml (T-75) or 10 ml (T182) of TrypLE and return to incubator for 5 to 15 minutes, or until cells detach.

Note: To avoid clumping DO NOT agitate the cells by hitting or shaking the flask while waiting for the cells to detach.

- 5) Add 3 to 5 ml (T-75) or 10 to 13 ml (T182) of complete growth medium and aspirate the cells by gentle pipetting.
- 6) Transfer the cell suspension to the centrifuge tube and centrifuge at approximately 1,300 rpm for 4 minutes. Discard the supernatant. Re-suspend the cell pellet in fresh growth medium. Perform 1:4 to 1:6 cells split as needed.

- 7) Incubate cultures at 37°C, 5% CO<sub>2</sub> humidified incubator.
- 8) Record each subculture event as a passage.

### **C. Maintenance and Generation of Seed Stocks**

- 1) Change media every 3-4 days thereafter. Use 25 ml (T-75) or 50 ml (T182) of growth medium.
- 2) Following first or second passage after receipt of cells and with sufficient number of cells to continue maintenance and expansion, the major portion of the flasks should be sub-cultured using TrypLE as above under “Sub-culture” and a small portion should be set aside as a seed stock. The cell pellet for the seed stock should be re-suspended in freezing medium.
- 3) Cells in freezing medium are dispensed into cryovials (1-5 million cells per 1 ml aliquot) and frozen in a -80°C cryo-freezing container overnight.
- 4) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

### **D. Harvest**

- 1) Detach cells from flasks as described above under “Sub-culture”.
- 2) Examine viability using Trypan blue staining and count cells. Transfer desired amount of cells to new tube and centrifuge and wash cells with PBS once for your application if needed.