

Human Hepatocellular Carcinoma (HepG2) Cells

HEPG2-500. Store cells at -80°C or in liquid nitrogen.

Product Description:

Human hepatocellular carcinoma cells were isolated from a liver biopsy of a 15 year old male caucasian donor; the donor had a well differentiated hepatocellular carcinoma. These cells were NOT isolated at CET but are sold to you under a unrestricted use license. HepG2 cells have been used to study a variety of phenomena including liver metabolism, cancer metastasis and tumor formation. These cells secrete a variety of major plasma proteins including albumin, α 2-macroglobulin, α 1-antitrypsin, transferrin, and plasminogen. HepG2 cells grow slow initially and tend to grow in clusters rather than as an intact monolayer.

Note: Once complete media has been formulated, it should be stored at 2-8°C. Avoid extended exposure of the medium to room or higher temperatures. Medium should be equilibrated in a water bath set at 37°C before adding media to any cell culture. Antibiotics/Antimycotics should not be used as an alternative to proper sterile technique.

Thawing and Plating HepG2 Cells

1. Remove the vial of cells from dry ice or storage unit. Defrost the vial of cells in a 37°C water bath with constant, moderate agitation, until ice in the ampoule is no longer visible.
2. Continue to warm the ampoule in the water bath for 30 seconds with gentle agitation.
3. Immediately disinfect the vial with 70% isopropanol.
4. Working in a laminar flow hood, open the vial and transfer the contents to a sterile 15 mL tube.
5. Very slowly, add approximately 10 mL of complete HepG2 Expansion Media (Table 1), pre-warmed to 37°C.
6. Centrifuge the suspended cells at 200 x g for 10 minutes.
7. Decant the medium and gently resuspend the pellet in the appropriate amount of media (Table 1) necessary to achieve a plating density of 20,000 cells/cm² of surface area.
8. At the end of 24 hours, aspirate media from the flask or dish, rinse 1X with Dulbecco's Phosphate Buffered Saline and replenish with fresh, pre-warmed HepG2 expansion media.
9. It is normal for HepG2 cells to grow slow initially, for a period of a week post-thaw. It is also normal for some cells to be shed during media changes. Lastly, HepG2 cells tend to grow in clusters rather than discrete monolayers.
10. Subculture cells at a 1:3 split ratio using 0.25% Trypsin/EDTA.

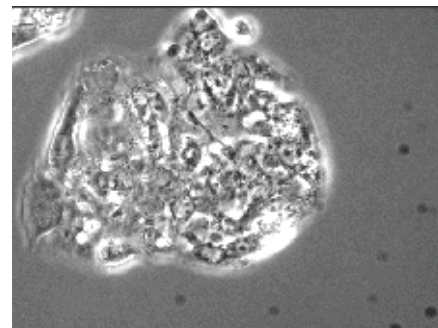


Figure 1. Human Hepatocellular Carcinoma HepG2 Cells.

Table 1: Preparation of 500 mL complete hepG2 Expansion Media

Brand	Amount for 500 mL	CET Product	Catalog Number
CET	450 mL	CET Hu. Hepatocellular Carcinoma Expansion Media	HEPG2.E.Media-450
Any	50 mL	Fetal Bovine Serum	Refer to Manufacturers' Catalog Number

Store Media at 4°C.

Certificate of Analysis

All hematopoietic, mesenchymal and multipotent stem cells are evaluated by flow cytometry for specific stem cell markers. All other cells are evaluated either by staining, method of isolation or traditional molecular biology techniques. Data is available upon request.

All growth and differentiation media are evaluated by conducting assays to make sure cells either grow or differentiate as indicated on the media label. Data is available upon request.

All cells are tested for HIV-1, HIV-2, Hepatitis B and Hepatitis C using sensitive PCR based assays. All cells test negative for these viruses. However, all human cells must be used in accordance with established laboratory safety procedures and only under the supervision of trained personnel.

Disclaimer

All products are for research use only. Not for diagnostic or therapeutic use. CET's products are designed and tested to function with other CET products only. For example, all of our cells are optimized to grow and differentiate in CET media. Although investigators are welcome to formulate their own media, CET cannot and will not guarantee that cells will function as indicated in the product brochure. Moreover, such third party use will void CET's obligation to replace cells, should they not function as indicated.

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Instructions for Use

- 1) If you do not plan on using the HepG2 cells right away, please store them minimally at -80°C and preferably in the vapor phase of a liquid nitrogen storage unit. HepG2 growth media should be stored at 4°C .
- 2) To use the cells, take the vial and thaw in a pre-heated 37°C water bath. As soon as no ice crystals are visible in the vial, wipe the vial with 70% ethanol, making sure no ethanol enters the vial.
- 3) Take the entire contents of the vial, approximately 1 milliliter, and aliquot this in a T-25 tissue culture dish containing 10 milliliters of pre-warmed complete HepG2 growth media. Complete HepG2 growth media is made by adding 50 milliliters of fetal bovine serum and 5 milliliters of Penicillin/Streptomycin.
- 4) Let cells incubate overnight at 37°C , 5% CO_2 with 90% relative humidity (standard tissue culture conditions).
- 5) The next day, aspirate off media carefully and add 10 milliliters of fresh, complete, HepG2 media, which has been pre-warmed to 37°C . Replace media every three days.
- 6) HepG2 cells tend to grow slowly at first and then very quickly. They also tend to cluster and grow vertically. This is normal.
- 7) When the tissue culture flask is confluent, split at 1:3 or 1:4 depending on how quickly you need your cells for subsequent experiments.



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All other cells are evaluated either by staining, method of isolation or traditional molecular biology techniques. Data is available upon request. All growth and differentiation media are evaluated by conducting assays to make sure cells either grow or differentiate as indicated on the media label. Data is available upon request.



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