



HepG2 Human Hepatocellular Carcinoma Expansion Media HEPG2.E.MEDIA-450. Store media at 4°C.

Media Usage Protocol:

HepG2 Human Hepatocellular Carcinoma Expansion Media is designed to be used with HepG2 Human Hepatocellular carcinoma cells, which are available separately. When used as directed, this media will support growth and expansion of these cells. The following is the recommended protocol for the usage of this media.

Note: Once complete media has been formulated, it should be stored at 2-8°C. Avoid extended exposure of the media to room or higher temperatures. Medium should be equilibrated in a water bath set at 37°C before adding media to any cell culture.

Additional Reagents Needed

1. Fetal Bovine Serum, High Grade or Characterized. Store in aliquots of 50mL at -20°C.
2. Penicillin/Streptomycin/Amphotericin C solution, 100X or Penicillin Streptomycin Solution, 100X. These solutions should be portioned in 5 mL aliquots, stored at -20°C. and never freeze/thawed.



Note: Antibiotics/Antimycotics should not be used as an alternative to proper aseptic technique

Formulating Complete HepG2 Expansion Media

1. Defrost 50mL of fetal bovine serum and 5 mL of antibiotic/antimycotic solution in a 37°C water bath until ice in the tubes is no longer visible.
2. Immediately disinfect the tubes and the bottle containing the base media with 70% isopropanol.
3. Working in a laminar flow hood, remove 5 mL of the media from the bottle and discard. This and all other procedures must be done in a sterile manner.
4. Add 50 mL of the fetal bovine serum to the base media.
5. Add 5 mL of the antibiotic/antimycotic solution to the base media.
6. Cap the bottle containing the now complete media and gently swirl a few times. The complete media is now ready to use. Store media at 4°C and prewarm to 37°C before use.

Using the Media

1. To use the HepG2 cells (available separately), 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.
2. Take the entire contents of the vial, approximately 1 milliliter, and aliquot this in a T-25 tissue culture dish containing 10 mL of pre-warmed complete HepG2 growth media.
3. Let cells incubate overnight at 37°C, 5% CO₂ with 90% relative humidity (standard tissue culture conditions).
4. 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.
5. HepG2 cells tend to grow slowly at first and then very quickly. They also tend to cluster and grow vertically. This is normal.
6. 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.

Table 1: Preparation of 500 mL of complete HepG2 Expansion Media

Brand	Amount for 500 mL	CET Product	Catalog #
CET	450 mL	CET HepG2 Expansion Media	HepG2.E.Media-450
Any	50 mL	Fetal Bovine Serum	Refer to Manufacturer's Catalog Number

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.