Cell Line Designation: Hep3B

AddexBio Catalog No. C0015001

Cell Line Description:

Disease: Hepatocellular carcinoma

Origin: Established from the tumor tissue of an 8-year-old black male

Species: Homo sapiens

Tissue: Liver

Properties: Adherent

Cytogenic data: Human hypotriploid stemline with near tetraploid side line

Patient: Male, 8 yrs of age

Complete Medium: AddexBio Formulated EMEM + 10% FBS

Subculture Procedure: 1:4 to 1:10 using 0.25% trypsin or trypsin/EDTA, 5% CO2; 37°C

Medium Renewal: Two to three times weekly.

Freezing Medium: Complete culture medium supplemented with 5% (v/v) DMSO

Additional Information: Additional product and technical information can be obtained from the catalog references and the Addexbio Technical Information site at www.addexbio.com, or by email at customersupport@addexbio.com.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. laboratory safety is discussed in the following publication: Biosafety in Microbiological and Biomedical Laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S.

Government Printing Office; 2007. The entire text is also available online at www.cdc.gov/od/ohs/biosafty/bmbl4/bmbl4toc.htm

Use Restrictions:

These cells are distributed for research purposes only. Addexbio does not recommend third party distribution of this cell line, as this practice has resulted in the unintentional spreading of contaminated cell lines.

Handling Procedure for Frozen Cells:

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

Safety Precaution:

Addexbio highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.

3. Transfer the vial contents to the centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125xg for 5 to 7 minutes. (Optional if one wants to remove DMSO)

4. Resuspend cell pellet with the recommended complete medium and dispense into a new culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6).

5. One may also transfer the vial contents into a new culture flask if removal of DMSO is not important. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6).

6. Incubate the culture at 37°C in a suitable incubator for 24-48 hours for cell attachment. A 5% CO_2 in air atmosphere is recommended.

Handling Procedure for Cells in Flask Culture:

The flask was seeded with cells grown and completely filled with complete medium at AddexBio facility that acts as a cushion and to prevent loss of cells during shipping.

1. Upon receipt, carefully examine if the majority of the cells are attached to the bottom of the flask using an inverted microscope (preferably equipped with phase-contrast optics), as the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable) during shipping. In addition, visually examine the culture for macroscopic evidence of any microbial contamination.
2. Spray lots of 70% Ethanol on the flask and the cap area. Carefully remove the parafilm and spray the cap area with ethanol again generously.
3. Place the flask erected on a flat surface inside of the biosafety cabinet.
4. Carefully unscrew the cap.
5. When preparing the cells for shipment, antibiotics were added to the medium.
6. It is recommended to culture the cells in antibiotics containing medium for the first week after arrival.
7. **For the cells that are still attached**, asceptically remove all but 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
8. **For the cells that are dettached**, in most of the case due to extended movement during shipment, cells detached from the flask. Asceptically remove the medium from the flask and centrifuge at 125 x g for 5 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to a new and sterile 25 cm^2 flask (T25). Incubate at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

References for Hep3B cells:

1. Wang, J., Wang, X. F., Zhang, L. G., Xie, S. Y., Li, Z. L., Li, Y. J., Li, H. H., and Jiao, F. Involvement of the mitochondrial pathway in p53-independent apoptosis induced by p28GANK knockdown in Hep3B cells. Cytogenet Genome Res, 125: 87-97, 2009.

2. Ji, L., Shen, K., Liu, J., Chen, Y., Liu, T., and Wang, Z. Intracellular glutathione regulates Andrographolide-induced cytotoxicity on hepatoma Hep3B cells. Redox Rep, 14: 176-184, 2009.

3. Yildirim, H. and Kockar, F. TGF-beta upregulates tumor-associated carbonic anhydrase IX gene expression in Hep3B cells. Cell Biol Int, 33: 1002-1007, 2009.

Lot Specific Information Sheet for AddexBio Cat #: C0015001

Lot Number: 0011125

Designation: Hep3B CELLS

Total Cells/mL: >1.5x10^6

Expected Viability: >76.5%

Ampule Passage #: 12

Dilute Ampule Content: 1:10 (T-25) or 1:15 (T-75)

Volume/Ampule: 1 mL

A T-25 setup at a dilution of 1:10, using culture medium as described in the product information sheet, reaches approximately 50-55% confluence within 24 to 48 hours.