Cell Line Designation: HepG2

AddexBio Catalog No. C0015002

Cell Line Description:

Disease: Hepatocellular carcinoma

Origin: Established from the tumor tissue of an 15-year-old Argentine boy

Species: Homo sapiens

Tissue: Liver

Properties: Adherent

Cytogenic data: Human hyperdiploid karyotype

Patient: Male, 15 yrs of age

Complete Medium: AddexBio-Formulated EMEM (C0005-01) + 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: 1

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 Cells Upon Arrival:

Frozen cells must be thawed immediately upon receipt and grown according to the handling procedures described here in this instruction manual to ensure the best cell viability.

Note: ***Avoid refreezing or repetitive freezing cells upon receipt*** *as it may result in irreversible damage to the cell line.*

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures described in this instruction manual.

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.

References for HepG2 cells:

1. Patlolla, A. K., Barnes, C., Hackett, D., and Tchounwou, P. B. Potassium dichromate induced cytotoxicity, genotoxicity and oxidative stress in human liver carcinoma (HepG2) cells. Int J Environ Res Public Health, 6: 643-653, 2009.

2. Souza, V., Escobar Mdel, C., Bucio, L., Hernandez, E., Gomez-Quiroz, L. E., and Gutierrez Ruiz, M. C. NADPH oxidase and ERK1/2 are involved in cadmium induced-STAT3 activation in HepG2 cells. Toxicol Lett, 187: 180-186, 2009.

3. Liu, Z. H. and Zeng, S. Cytotoxicity of ginkgolic acid in HepG2 cells and primary rat hepatocytes. Toxicol Lett, 187: 131-136, 2009.

4. Zhang, X., Cao, J., Jiang, L., Geng, C., and Zhong, L. Protective effect of hydroxytyrosol against acrylamide-induced cytotoxicity and DNA damage in HepG2 cells. Mutat Res, 664: 64-68, 2009.

5. Verma, P., Verma, V., Ray, P., and Ray, A. R. Agar-gelatin hybrid sponge-induced three-dimensional in vitro 'liver-like' HepG2 spheroids for the evaluation of drug cytotoxicity. J Tissue Eng Regen Med, 3: 368-376, 2009.

6. Barcelos, G. R., Angeli, J. P., Serpeloni, J. M., Rocha, B. A., Mantovani, M. S., and Antunes, L. M. Effect of annatto on micronuclei induction by direct and indirect mutagens in HepG2 cells. Environ Mol Mutagen, 50: 808-814, 2009.

Lot Specific Information Sheet for AddexBio Cat #: C0015002

Lot Number: 0038272

Designation: HepG2 CELLS

Total Cells/mL: >$8.5x10^6$

Expected Viability: >85.5%

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.

Remarks:

Hep G2 cells initially attach in small patches of cells with many clusters still in suspension. After a few days, growth will extend outward from the adherent cell colonies. Occasionally, the cells will pile on the adherent colonies forming a multilayered appearance, which is not an unusual morphology for this cell line. During the first week of recovery from cryopreservation, there are normally viable floating cells in the culture. Do not discard the floating cells, they should be retained by gentle centrifugation (125 xg) and added back to the adherent population, when feeding with fresh medium every 2 or 3 days. Separating or discarding the viable floating cells can make the culture too dilute and growth will lag (or stop).

Subtle changes in culture conditions, particularly in pH and the quality of serum used in the growth medium, may affect the amount of clumping exhibited by the cells. The cells also tend to be vacuolar, especially at confluence. The complete growth medium should be supplemented with a high quality, low endotoxin, fetal bovine serum that has not been heat-inactivated. Using such serum, may help the rounded cell clusters adhere better and flatten into a single monolayer.