Rev. 1

Human Hepatocytes (HH)

Catalog #5200-2

Cell Specification

The liver is critical for many vital functions including: energy metabolism, biotransformation of xenobiotics, synthesis of plasma proteins under physiological and pathophysiological conditions, and detoxification of substances [1]. Human Hepatocytes (HH) are multifunctional cells that produce proteins required for protein synthesis, synthesize cholesterol, store proteins, produce and secrete bile, and detoxify substances [1-3]. Cultured HH are an excellent *in vitro* model for studying liver function, metabolism, and liver disease. Understanding the molecular mechanisms of the liver may help to elucidate new therapies for treatment of hepatic disease.

HH from ScienCell Research Laboratories are isolated from human liver. HH are cryopreserved immediately after purification and delivered frozen. Each vial contains >2 x 10^6 cells in 1 ml volume. HH are characterized by immunofluorescence with antibodies specific to cytokeratin-18 and/or albumin. HH are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. ***HH are not recommended for expanding or long term cultures since the cells do not proliferate in culture***.

Recommended Medium

It is recommended to use Hepatocyte Medium (HM, Cat. #5201) for the culturing of HH *in vitro*.

Product Use

HH are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[1] Runge D, Michalopoulos GK, Strom SC, Runge DM. (2000) "Recent advances in human hepatocyte culture systems." *Biochem. Biophysi. Res. Comm*. 274:1-3.

[2] Chen HL, Wu HL, Fon CC, Chen PJ, Lai MY, Chen DS. (1998) "Long-term culture of hepatocytes from human adults." *J. Biomed. Sci*. 5:435-440.

[3] Fitzpatrick E, Mitry RR, Dhawan AJ. (2009) "Human hepatocyte transplantation: state of the art." *Intern. Med.* 266:339-57.

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Instructions for culturing cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37°C water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Note: Experiments should be well organized before thawing cells, as hepatocytes do not proliferate in culture. It is recommended that HH are used for experiments as quickly as possible after thawing the cells.

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37°C, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare two poly-$_L$-lysine-coated 6-well cell culture plates (2 µg/cm^2). For example, add 2 ml of sterile water to one well of a 6-well plate and then add 20 µl of poly-$_L$-lysine stock solution (1 mg/ml, Cat. #0403); repeat procedure for additional wells. Leave the vessel in a 37°C incubator overnight (or for a minimum of one hour).
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.
3. Rinse the poly-$_L$-lysine-coated vessel twice with sterile water and then add 3 ml of complete medium into each well. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-$_L$-lysine-coated culture vessel. Distribute evenly in each well of the two 6-well plates. A seeding density of 20,000 cells/cm^2 is recommended.

   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important that cells are plated in poly-$_L$-lysine-coated culture vessels to promote cell attachment.
6. Replace the lid of the culture vessel and ensure that the cells are evenly distributed.
7. Return the culture plates to the incubator.
8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells.
9. Use cells promptly for experiments.

Note: HH cannot be subcultured or passaged since this cell type will terminally differentiate in long term culture.

Note: We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

Caution: Handling human derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Culture Methods*. 11: 191-9.