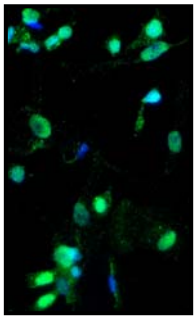
REF: P10671

HEPATIC CELL SYSTEM INNOPROFILE™

HUMAN LIVER MACROPHAGES – HUMAN KUPFFER CELLS



Product Type:	Cryo-preserved Kupffer Cells
Catalog Number:	P10671
Source:	Human Liver
Number of Cells:	1 x 10^6 Cells / vial (1ml)
Storage:	Liquid Nitrogen

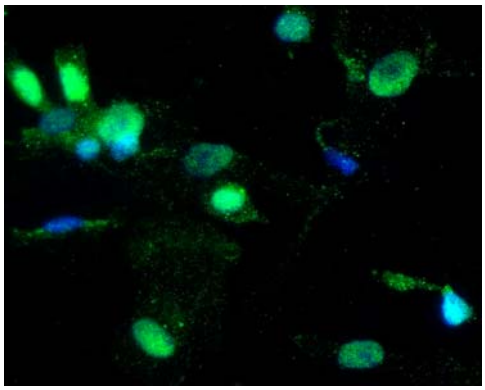
Human Kupffer Cells (HKC) provided by Innoprot are isolated from human healthy liver. Human Kupffer Cells are cryopreserved immediately after purification and delivered frozen. HKC are guaranteed to further culture in the conditions provided in this technical sheet; however, HKC are not recommended for expanding or long-term cultures since the cells do not proliferate in regular culture.

Human Liver Macrophages, which are also known as Kupffer cells, reside within the lumen of liver sinusoids. HKC protect the liver by responding to pathogens and metastatic cells, while tolerating harmless self and foreign antigens, which enter via blood flow through the portal vein and hepatic artery. Recent studies have shown that kupffer cells play an important role in fibrosis, liver inflammation, fatty liver disease, and liver transplantation. HKC are an excellent model for studying macrophage functions under normal physiological and pathological conditions.

Recommended Medium

- Macrophage Medium

(Reference: P60136)



Product Characterization

Immunofluorescent method

- F4/80

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi

Product Use

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures

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INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: Cryopreserved cells are very delicate. Thaw the vial in a 37 °C waterbath and return them to culture as quickly as possible with minimal handling!
Experiments should be well organized before thawing Kupffer cells. It is recommended that Kupffer cells are used for experiments as quickly as possible after thawing the cells since they ***cannot be subcultured or passaged***, as ***the cells do not proliferate***.

Set up culture after receiving the order:

1. Prepare a poly-L-lysine-coated culture plate (2µg/cm² is recommended). For example, add 2 ml of sterile water to one well of a 6-well plate and then add 20 ul of poly-L-lysine stock solution (1 mg/ml, Cat. #PLL). Leave the plate in a 37°C incubator overnight (minimum two hours).

2. Prepare complete medium (MaM, Cat. #P60136). Thaw MaGS, FBS and P/S solution at 37°C. Gently tilt the tubes several times to ensure the contents are completely mixed before adding to the medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. In a sterile field, remove the caps without touching the interior threads with fingers. Add MaGS, FBS and P/S solution to the medium and mix well.

3. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add the volume of complete medium recommended in Table 1 or Table 2. Leave the plate(s) 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.

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.

5. Carefully remove the cap without touching the interior threads and gently resuspend the cell suspension. A seeding density of 10,000-20,000 cells/cm² is recommended depending on your experiments. We recommend following Table 1 for seeding HKC onto 6-well, 12-well, or 24-well plates. For seeding HKC on 60 mm plates, use Table 2.

6. Pipet the correct volume of cell suspension into each well of an equilibrated, poly-L-lysine-coated culture plate containing complete medium. Replace the lid of the culture plate and gently rock the plate to distribute the cells evenly.

7. Return the culture plate to the incubator.

8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the culture medium the next morning after establishing a culture from cryopreserved cells to remove residual DMSO and unattached cells. Once the macrophages attach, the cells can be used for experiments.

9. Use cells promptly for experiments.

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Table 1
Recommended cell suspension volume per vial using a 6-well, 12-well, or 24 well format

Well format	Surface area/well (approx. values)	Volume of media/well	Volume of cell suspension from vial/well	# of wells/vial
6-well	9.6 cm^2	3.0 ml	150 µl	6 wells
12-well	3.9 cm^2	2.0 ml	60 µl	15 wells
24-well	1.9 cm^2	1.0 ml	30 µl	30 wells

Table 2
Recommended cell suspension volume per vial using 60 mm plates

Plate Format	Surface area/plate (approx. values)	Volume of cell suspension from vial/plate	# of plates/vial	Volume of media (ml)/plate
60 mm	21 cm^2	300 µl	3	3.0 ml

Caution: *Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working 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, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. *J Tissue Culture Methods.* 11(4).

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