User Guide

Normal Human Hepatic Kupffer Cells

HLP400-250K HLP400-500K HLP400-1M

Store in liquid nitrogen

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for Human or Animal Consumption

Product Overview

Kupffer Cells, also known as Browicz-Kupffer cells and stellate macrophages, are specialized macrophages located in the liver lining the walls of the sinusoids. These cells are constantly exposed to gut-derived bacteria, microbial debris and bacterial endotoxins, known to activate macrophages. Upon activation Kupffer cells release various products, including cytokines, prostanoides, nitric oxide and reactive oxygen species. These factors regulate the phenotype of Kupffer cells themselves, and the phenotypes of neighboring cells, such as hepatocytes, stellate cells, endothelial cells and other immune cells that traffic through the liver. Therefore, Kupffer cells are intimately involved in the liver's response to infection, toxins, ischemia, resection, and other stresses. Human Hepatic Kupffer Cells are obtained via the gift of organ donation. Each lot is guaranteed for post thaw cell viability of ≥70%. Kupffer Cells can be characterized using flow cytometry for population distributions, and are positive for CD11b, CD14, and CD68 (Seiki *et al*., 2014).

Seki S, Ikarashi M, Kinoshita M, Nakashima M and Nakashima H. New Findings about Liver Kupffer Cells/Macrophages, B Cells and their Functions. J Hepat Res. 2014;1(1): 1003.

Quality Control Testing

- Post-thaw viability of ≥70%, with a yield of ≥250 K, 500 K, or 1 M viable cells per vial.
- Cell surface marker analysis: CD68, CD11b, CD14, and CD45.
- Cytokine production by Kupffer Cells following 100ng/ml LPS stimulation - IL6.
- Each donor is tested negative for: HIV, Hepatitis B, Hepatitis C, and syphilis*.
- The culture is tested negative for: Gram +, Gram -, Mycoplasma and Fungi.

*No known test can offer complete assurance that the viruses that cause HIV-1, HIV-2, HTLV I, HTLV II, hepatitis B and hepatitis C are not present. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. All human based products should be handled at a BSL-2 (Biosafety Level 2) or higher.

Materials Provided

Normal Human Hepatic Kupffer Cells:

One (1) vial containing 250 K, 500 K or 1 M cells per vial

Materials Required (Not provided)

Catalog numbers in ( ) can be ordered from SigmaAldrich.com unless otherwise noted

- Collagen Type I, Rat Tail (08-115)
- Tissue culture treated multi-well plates or tubes
- Please see Protocol for media components

Storage and Stability

Upon receipt, immediately store cryovial(s) in vapor phase liquid nitrogen.

Protocols

All protocols are performed within a Class II laminar flow biohood and with an aspirator unless otherwise specified. Incubators are humidified and are set to 37 °C and 5% CO_2. PPE should be worn such as gloves, lab coat, and safety glasses.

Preparing Collagen Coated Plate or Culture Ware

1. Dilute the collagen to a final concentration of 56 µg/mL in sterile 70% ethanol and gently mix until the collagen is solubilized.
2. Add the appropriate volume of the collagen/ethanol mixture to each well to completely cover the bottom of wells or flasks.
3. Gently move the cell culture plate until the collagen/ethanol mixture evenly coats the inside of the wells or flasks.
4. Air dry plates or flasks in a laminar flow hood. Leave cell culture plate or flask over night with the cover or the cap ajar to allow airflow and prevent condensation.

Preparing Kupffer Cell Media

Formulations for Kupffer cell media are readily available from literature. Below is an example media from one of the publications*. All components listed below are available at SigmaAldrich.com.

Components	Catalog Number	Working Stock	Final Dilution	Final Conc.	Final Volume (mL)
Williams E	W1878-500ML			1 x	470
FBS	ES-009-B			5%	25
Insulin	I9278-5ML	10 mg/mL (1.7 mM)	1700 x	1 uM	0.29
Pen/Strep	P7539	100 x	100 x	1 x	5
				Total Volume	**500**

* Activation of human and mouse Kupffer cells by lipopolysaccharide is mediated by CD14.

Grace L. Su, Sanna M. Goyert, Ming-Hui Fan, Alireza Aminlari, Ke Qin Gong, Richard D. Klein, Andrzej Myc, William H. Alarcon, Lars Steinstraesser, Daniel G. Remick, and Stewart C. Wang American Journal of Physiology-Gastrointestinal and Liver Physiology 2002 283:3, G640-G645

Thawing and Plating Cryopreserved Kupffer Cells

Before starting, please ensure collagen I coated culture ware is prepared.

1. **DO NOT** pre-warm medium to thaw cells. Kupffer cells easily attach to the walls of the conical tube at 37 °C. Therefore, use of pre-warmed media is not recommended.
2. Place vial in a 37 °C water bath, hold and rotate vial gently until the contents are completely thawed. Remove the vial from the water bath immediately, wipe dry, rinse the vial with 70% ethanol and transfer to a sterile field. Remove cap, being careful not to touch the interior threads with fingers.
3. Using a pipette, gently transfer contents of vial to a 15 mL conical tube containing 9 mL of COLD (4 °C) Kupffer Cell Medium and place the tube on ice.
4. Centrifuge tube at 500 x *g* for 5 minutes. After centrifugation, aspirate medium and re-suspend cell pellet in 1 mL **COLD** Kupffer Cell Medium.

   Note: the pellet will be very small. Resuspend using a P1000 micropitetter, as resuspension using a serological pipette may lead to clumping of the cells.
5. Count the cells using the trypan blue exclusion assay.
6. Dilute the cells in **WARM** Kupffer Cell Medium to 400,000 cells/mL.
7. Plate 100,000 cells/cm^2 on culture ware coated with collagen type I (Refer to the table below).

Format	cm^2 Per Well	cm^2 Per cm^2	Cells Per Well	Total Cells Per Plate
384	0.056	100,000	5,600	2.15 x 10^6
95	0.32	100,000	32,000	3.07 x 10^6
48	1.02	100,000	102,000	4.89 x 10^6
24	1.94	100,000	194,000	4.66 x 10^6
12	3.87	100,000	387,000	4.64 x 10^6
6	9.62	100,000	962,000	5.77 x 10^6
10 cm dish	56	100,000	---	5.60 x 10^6
60 mm dish	21	100,000	---	2.10 x 10^6
35 mm dish	8	100,000	---	800,000
T-25	25	100,000	---	2.5 x 10^6
T-75	75	100,000	---	7.5 x 10^6

8. Place the cells in a humidified 37 °C/5% CO^2 incubator and allow them to attach for 4-6 hours to overnight.

   Note: Human Kupffer cells can be removed easily using vacuum aspiration. Use caution.
9. After attachment, replace the medium with fresh WARM Kupffer Cell Medium.
10. After 24 hours, replace the medium with warmed Kupffer Cell Medium and proceed with your experiment.
    Note: These cells DO NOT proliferate and cannot be passaged.

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