OptiPrep™ Application Sheet C47

Enrichment of hepatic Kupffer cells in a discontinuous gradient

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- **The Axis-Shield Mini-Review "Hepatic non-parenchymal cells" (MC08)** compares some of the methodologies and provides a comprehensive bibliography of all the published papers reporting the use of OptiPrep™ for the isolation of Kupffer cells
- **MC10 "Hepatic non-parenchymal Kupffer and sinusoidal endothelial cells (and other liver cell types) – a bibliography** provides a comprehensive bibliography of all the published papers reporting the use of OptiPrep™ for the isolation of these cells
- To access return to the initial list of Folders and select "**Mini-Reviews**".
- To access other Application Sheets referred to in the text return to the Cell Index; key Ctrl "F" and type the C-Number in the Find Box

1. Background

Parenchymal and non-parenchymal cells (PC and NPC) may be prepared by collagenase digestion of the liver using a tissue perfusion system. The PCs are then separated from the NPC by differential pelleting at 50 *g* for 1-4 min. It is however necessary to repeat this centrifugation (maybe twice more) to remove PC from the supernatant; moreover the NPC yield is usually low. It is both more common and more effective to carry out the 50 *g* centrifugation once; to harvest all the cells from the supernatant by centrifugation at a higher *g*-force and then use a density barrier prepared from one of the of iodinated density gradient media to resolve the two types of cell. Many workers prefer a modified perfusion strategy; it uses a mixture of collagenase and Pronase or *Clostridium perfringens* enterotoxin to destroy the PC selectively [1,2]. Hendriks et al [3] preferred Pronase because of the uncertain commercial availability of the enterotoxin and the latter's possible cause of cell blebs.

One- or two-layer density gradient centrifugation alone may not be sufficiently discriminating to provide a pure preparation of Kupffer cells, but this technique can provide an important initial enrichment for these cells prior to the use of centrifugal elutriation, adherence of the Kupffer cells to a plastic surface; sometimes both elutriation and surface adherence are used. Antibody-bound magnetic beads have also been used as a final purification step. See Section 5 for more information about additional procedures. The methods in this Application Sheet may simply provide a pure preparation of total NPC or of a NPC fraction also impoverished in the lighter stellate cells (see Section 4). However multiple-layer flotation gradients may be able achieve an improved resolution of Kupffer cells from other NPC types – see Section 4c.

2. Solution selection and preparation

The solution used to suspend the crude NPC suspension and to dilute the OptiPrep™ may be a routine buffered saline such as PBS [4,5], which may be supplemented with 1% BSA [6] or a balanced salt solution such as Hank's Balanced Salt Solution (HBSS) [7-11] or an NPC customized medium such as Gey's balanced salt solution [12-15] and this may be prepared as described in the box. In a few instances a commercial culture medium is used, such as F-12 [16] or RPMI, which may be supplemented with 1% BSA [17] or 10% FCS [18,19]. Only when the solution contains 10% serum is the density of a culture medium likely to be significantly different to PBS, HBSS or GBSS (i.e. approx 1.006 g/ml). A medium containing 10% serum has a density of approx 1.009 g/ml.

GBSS
Dissolve the following in 500 ml water:
7.0 g NaCl
0.37 g KCl
70 mg $MgSO_4.7H_2O$
150 mg $Na_2HPO_4.2H_2O$
220 mg $CaCl_2.2H_2O$
2.27 g $NaHCO_3$
30 mg KH_2PO_4
210 mg $MgCl_2.6H_2O$
1.0 g glucose

Make up to 1 liter of water and gas with 5% CO_2/air. The pH should be 7.4.

- For more information on the preparation of gradient solutions **see Application Sheet C01**.

3. Species source

The species source for the liver cells may very well influence the detailed methods used in the pre-gradient stages such as perfusion of the liver, enzymic and physical disaggregation of the tissue and washing of the released cells. There are some significant differences in the density gradient methodology used in preparing the Kupffer cell-enriched fraction, but whether any of this is species related is not known. Most papers report the use of either rat [6-12, 20-23] or mouse liver [5, 13-16, 18, 24, 25], but pig [4, 17, 26, 27] and human [28] are also used as sources.

4. Protocols

Note that in many published methods the gradients are described in terms of % OptiPrep™; often this is % (v/v) OptiPrep™. Sometimes however it is actually % (w/v) iodixanol; i.e. iodixanol and OptiPrep™ are used synonymously, which is incorrect (OptiPrep™ is the commercial name for a solution of 60% (w/v) iodixanol). In the following text all gradient solutions are given as % (w/v) iodixanol.

4a. Flotation from a density-adjusted cell suspension

This is the simplest strategy in which the crude NPC suspension is mixed with OptiPrep™ to a certain concentration of iodixanol; a small volume of saline or balanced salt solution (2-3 ml) is layered on top and centrifuged. The NPC float to the interface with the saline and any PC, residual erythrocytes, non-viable cells or cell fragments either pellet or remain suspended in the load zone. Some examples are given in Table 1. Although some workers omit the upper layer, its presence is recommended since it prevents the cells banding at an air/liquid interface. Most centrifugations are at 4°C.

Table 1 Flotation from a density-adjusted cell suspension

Cell suspension adjusted to:	Centrifugation	Secondary purification	Ref. No
12-12.6% (w/v) iodixanol	3,300 *g* – 30 min	Elutriation OR adherence	4, 17,26,27
10.2% (w/v) iodixanol (6 ml)	1,600 *g* – 17 min	Adherence	13-15
10.8% (w/v) iodixanol	1,500 *g* –20 min	Adherence	11

4b. Two-density layer sedimentation

The crude NPC preparation in HBSS is adjusted to 11.7% (w/v) iodixanol (approx ρ = 1.066 g/ml); layered over a solution of 17.6% (w/v) iodixanol (approx ρ = 1.097 g/ml) and overlaid by HBSS. After centrifugation at 1400 *g* for 17 min at 4°C NPC banded at the top and bottom of the 11.7% iodixanol layer; both layers were further processed by elutriation or adherence [7]. This configuration was also used by Yang et al [29]. In a slight modification of this two-layer gradient, the cell suspension was layered over the two iodixanol solutions rather being adjusted to the lower density [8-10, 20-22, 25]. Park et al [16] used a similar strategy layering the crude cell suspension over 8.2% and 15.6% (w/v) iodixanol; while der Flier et al [30] increased the density of the lower layer to 17.6%. More recently Hyun et al [31] separated the stellate and Kupffer + endothelial cells using 11.5% and 20% (w/v) iodixanol.

4c. Multiple layer gradients - flotation

Schreiber et al [32] diluted OptiPrep™ with Krebs-Henseleit buffer containing 1.25 mM $CaCl_2$ and 1.2 mM Na_2SO_4 to produce solutions of 17%, 11.5% and 8.4% (w/v) iodixanol. The crude NPCs were suspended in 24% iodixanol; this was overlaid by the lower density solutions and finally the buffer. After centrifugation at 1,400 *g* for 20 min at 4°C, the stellate and Kupffer cells banded predominantly at the interface of the buffer/8.4% and 8.4/11.5% interfaces respectively.

4d. Removal of debris

Sometimes a quite dense solution of 24% (w/v) iodixanol is used as a barrier merely to remove debris and non-viable cells, which tend to be denser than the Kupffer cells [5, 18, 33]. This can also be achieved simply by adjusting the cell suspension to approx 26% (w/v) iodixanol and after layering a small volume of HBSS on top, centrifuging at 400 *g* for 15 min and collecting the cells from the interface [19].

5. Add-on procedures

To include elutriation schedules is beyond the scope of this Application Sheet, but some of the commonly used adherence methods can be briefly summarized: either dishes coated with glutaraldehyde-fixed bovine serum albumin [4, 6, 26] or collagen have been used [13, 14, 16]. Antibody-bound bead techniques were reported in refs 5, 18, 19, 24, 33 and 34.

6. References

1. Boyum, A., Berg, T. and Blomhoff, R. (1983) *Fractionation of mammalian cells* In Iodinated density gradient media – a practical approach (ed. Rickwood, D.) IRL Press at Oxford University Press, Oxford, UK, pp 147-171
2. Brouwer, A., Hendricks, H. F. J., Ford, T. and Knook, D. L. (1991) *Centrifugation separations of mammalian cells* In Preparative centrifugation – a practical approach (ed. Rickwood, D.) IRL Press at Oxford University Press, Oxford, UK, pp 271-314
3. Hendriks, H.F.J., Brouwer, A. and Knook, D.L. (1990) *Isolation, purification, and characterization of liver cell types* Methods Enzymol., **190**, 49-58
4. Elvevold, K., Nedredal, G.I., Revhaug, A., Bertheussen, K. And Smedsrød, B. (2005) *Long-term preservation of high endocytic activity in primary cultures of pig liver sinusoidal endothelial cells* Eur. J. Cell Biol., **84**, 749-764
5. Shao, B., Lu, M., Katz, S.C., Varley, A.W., Hardwick, J., Rogers, T.E., Ojogun, N., Rockey, D.C., DeMatteo, R.P. and Munford, R.S. (2007) *A host lipase detoxifies bacterial lipopolysaccharides in the liver and spleen* J. Biol. Chem., **282**, 13726-13735
6. Malerød, L., Juvet, L.K., Gjøen, T. and Berg, T. (2002) *The expression of scavenger receptor class B, type I (SR-BI) and caveolin-1 in parenchymal and nonparenchymal liver cells* Cell Tissue Res., **307**, 173-180
7. Valatas, V., Xidakis, C., Roumpaki, H., Kolios, G. and Kouroumalis, E.A. (2003) *Isolation of rat Kupffer cells: a combined methodology for highly purified primary cultures* Cell Biol Int., **27**, 67-73
8. Xidakis, C., Ljumovic, D., Manousou, P., Notas, G., Valatas, V., Kolios, G., and Kouroumalis, E. (2005) *Production of pro- and anti-fibrotic agents by rat kupffer cells; the effect of octreotide* Digest. Dis. Sci., **50**, 935-941
9. Charalampopoulos, I., Androulidaki, A., Minas, V., Chatzaki, E., Tsatsanis, C., Nota, G., Xidakis, C., Kolios, G., Kouroumalis, E., Margioris, A.N. and Gravanis, A. (2006) *Neuropeptide urocortin and its receptors are expressed in rat Kupffer cells* Neuroendocrinology, **84**, 49-57
10. Xidakis, C., Mastrodimou, N., Notas, G., Renieri, E., Kolios, G., Kouroumalis, E. and Thermos, K. (2007) *RT-PCR and immunocytochemistry studies support the presence of somatostatin, cortistatin and somatostatin receptor subtypes in rat Kupffer cells* Regul. Pept., **143**, 76-82
11. Baranova, I.N., Bocharov, A.V., Vishnyakova, T.G., Kurlander, R., Chen, Z., Fu, D., Arias, I.M., Csako, G., Patterson, A.P. and Eggerman, T.L. (2010) *CD36 is a novel serum amyloid A (SAA) receptor mediating SAA binding and SAA-induced signaling in human and rodent cells* J. Biol. Chem., **285**, 8492–8506
12. DeLeve, L.D., Wang, X., McCuskey, M.K. and McCuskey, R.S. (2006) *Rat liver endothelial cells isolated by anti-CD31 immunomagnetic separation lack fenestrae and sieve plates* Am. J. Physiol. Gastrointest. Liver Physiol., **291**, G1187-G1189
13. Hu, S., Yin, S., Jiang, X., Huang, D. and Shen, G. (2009) *Melatonin protects against alcoholic liver injury by attenuating oxidative stress, inflammatory response, and apoptosis* Eur. J. Pharmacol., **616**, 287–292
14. Hu, S., Shen, G., Zhao, W., Wang, F., Jiang, X. and Huang, D. (2010) *Paeonol, the main active principles of Paeonia moutan, ameliorates alcoholic steatohepatitis in mice* J. Ethnopharmacol., **128**, 100–106
15. Lv, X., Chen, Z., Li, J., Zhang, L., Liu, H., Huang, C. and Zhu, P. (2010) *Caffeine protects against alcoholic liver injury by attenuating inflammatory response and oxidative stress* Inflamm. Res., **59**, 635–645
16. Park, J.K., Cho, K., Johnson, J. and Perez, R.V. (2004) *Induction of MIP-1α in Kupffer cell by portal venous transfusion* Transplant Immunol., **13**, 33-38
17. Nedredal, G.I., Elvevold, K.H., Ytrebø, L.M., Olsen, R., Revhaug, A. and Smedsrød, B. (2003) *Liver sinusoidal endothelial cells represent an important blood clearance system in pigs* Comp. Hepatol., **2:**1
18. Burt, B.M., Plitas, G., Stableford, J.A., Nguyen, H.M., Bamboat, Z.M., Pillarisetty, V.G. and DeMatteo, R.P. (2008) *CD11c identifies a subset of murine liver natural killer cells that responds to adenoviral hepatitis* J. Leukoc. Biol., **84**, 1039-1046
19. Connolly, M.K., Bedrosian, A.S., Malhotra, A., Henning, J.R., Ibrahim, J., Vera, V., Cieza-Rubio, N.E., Hassan, B.U., Pachter, H.L., Cohen, S., Frey, A.B. and Miller, G. (2010) *In hepatic fibrosis, liver sinusoidal endothelial cells acquire enhanced immunogenicity* J. Immunol., **185**, 2200–2208
20. Valatas, V., Kolios, G., Manousou, P., Notas, G., Xidakis, C., Diamantis, I. and Kouroumalis, E. (2004) *Octreotide regulates CC but not CXC LPS-induced chemokine secretion in rat Kupffer cells* Br. J. Pharm., **141**, 477-487
21. Valatas, V., Kolios, G., Manousou, P., Xidakis, C., Notas, G., Ljumovic, D. and Kouroumalis, E.A. (2004) *Secretion of inflammatory mediators by isolated rat Kupffer cells; the effect of octreotide* Regul. Pept., **120**, 215-225
22. Kolios, G., Valatas, V., Manousou, P., Xidakis, C., Notas, G. and Kouroumalis, E. (2008) *Nitric oxide and MCP-1 regulation in LPS activated rat Kupffer cells* Mol. Cell. Biochem., **319**, 91-98
23. Xie, G., Wang, L., Wang, X., Wang, L. and DeLeve, L.D (2010) *Isolation of periportal, midlobular, and centrilobular rat liver sinusoidal endothelial cells enables study of zonated drug toxicity* Am. J. Physiol. Gastrointest. Liver Physiol., **299**, G1204–G1210
24. Zhu, J., Huang, X. and Yang, Y. (2007) *Innate immune response to adenoviral vectors is mediated by both toll-like receptor-dependent and -independent pathways* J. Virol., **81**, 3170-3180

25. Meng, Z., Fu, X., Chen, X., Zeng, S., Tian, Y., Jove, R., Xu, R. and Huang, W. (2010) *miR-194 is a marker of hepatic epithelial cells and suppresses metastasis of liver cancer cells in mice* Hepatology, **52**, 2148-2157
26. Elvevold, K.H., Nedredal, G.I., Revhaug, A. and Smedsrød, B. (2004) *Scavenger properties of cultivated pig liver endothelial cells* Comp. Hepatol., **3:**1
27. Nedredal, G.I., Elvevold, K., Ytrebø, L.M., Fuskevåg, O-M., Pettersen, I., McCourt, P.A., Bertheussen, K., Smedsrød, b. and Revhaug, A. (2009) *Porcine liver sinusoidal endothelial cells contribute significantly to intrahepatic ammonia metabolism* Hepatology, **50**, 900-908
28. Wallace, K., Cowie, D.E., Konstantinou, D.K., Hill, S.J., Tjelle, T.E., Axon, A., Koruth, M., White, S.A., Carlsen, H., Mann, D.A. and Wright, M.C. (2010) *The PXR is a drug target for chronic inflammatory liver disease* J. Steroid Biochem. Mol, Biol., **120**, 137–148
29. Yang, H., Tong, C., Fu, C., Xu, Y., Liu, X., Chen, Q., Zhang, Y., Lü, S., Li, N. and Long, M. (2016) *Analyses of movement and contact of two nucleated cells using a gas-driven micropipette aspiration technique* J. Immunol. Meth., **428**, 20–29
30. Van der Flier, A., Liu, Z., Tan, S., Chen, K., Drager, D., Liu, T., Patarroyo-White, S., Jiang, H. and Light, D.R. (2015) *FcRn rescues recombinant factor VIII Fc fusion protein from a VWF independent FVIII clearance pathway in mouse hepatocytes* PLoS One, **10**: e0124930
31. Hyun, J., Wang, S., Kim, J., Rao, K.M., Park, S.Y., 2, Chung, I., Ha, C-S., Kim, S-W., Yun, Y.H. and Jung, Y. (2016) *MicroRNA-378 limits activation of hepatic stellate cells and liver fibrosis by suppressing Gli3 expression* Nat. Comm., **7**: 10993
32. Schreiber, R., Taschler, U., Wolinski, H., Seper, A., Tamegger, S.N., Graf, M., Kohlwein, S.D., Haemmerle, G., Zimmermann, R., Zechner, R. and Lass, A. (2009) *Esterase 22 and beta-glucuronidase hydrolyze retinoids in mouse liver* J. Lipid Res., **50**, 2514–2523
33. Connolly, M.K., Mallen-St. Clair, J., Bedrosian, A.S., Malhotra, A., Vera, V., Ibrahim, J., Henning, J., Pachter, H.L., Bar-Sagi, D., Frey, A.B. and Miller, G. (2010) *Distinct populations of metastases-enabling myeloid cells expand in the liver of mice harboring invasive and pre-invasive intra-abdominal tumor* J. Leukoc. Biol., **87**, 713–725
34. Katz, S.C., Pillarisetty, V.G., Bleier, J.I., Shah, A.B. and DeMatteo, R.P. (2004) *Liver sinusoidal endothelial cells are insufficient to activate T cells* J. Immunol., **173**, 230-235

Application Sheet C50; 4th edition, June 2016

AXIS-SHIELD
Innovation for Life