OptiPrep™ Application Sheet C28

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
- **Application Sheet C25 "Hepatic non-parenchymal cells (stellate, Kupffer and endothelial) cells – a short methodological survey"** compares some of the methodologies for these cells.
- **OptiPrep™ Reference List RC08 "Hepatic non-parenchymal, Kupffer and sinusoidal endothelial cells (and other liver cell types)"** provides a comprehensive list 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 "**Reference Lists**".
- 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

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