Excellence in Separations

OptiPrep™ Application Sheet C22

Isolation of dendritic cells from tissues by a mixer technique

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- The **OptiPrep™ Reference List RC05** "**Dendritic cells from blood and tissues**" compares all of the current methodologies and provides a full bibliography of all the published papers reporting the use of OptiPrep™: 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

Since dendritic cells (DC) were recognized as playing an important role in the induction of cell-mediated responses [1], there has been a rapid growth in research into the function of these cells and methods for their purification. Gradients of either albumin or metrizamide, although providing an effective enrichment of DC, tended to cause some functional alteration of the cells (see ref 2 for details). However, because cells are much more tolerant of Nycodenz®, this iodinated density gradient medium rapidly became established as the medium of choice for DC cell purification from peripheral blood and from lymphoid tissues. More recently methods have been developed for isolation of DC from tissues in iodixanol gradients because of the even higher tolerance of this solute by cells.

Compared to other cell types in tissues such as spleen, thymus, lymph nodes etc., the DCs have a low density and one of the most commonly Nycodenz®-based techniques is simply to isolate low-density cells by layering the disaggregated cell suspension over a barrier of density 1.077-1.080 g/ml (e.g. refs 3-5). This sedimentation strategy has also been used with OptiPrep™ and it is described in **"Dendritic cells" Application Sheet C41, see index.**

Another common approach is to suspend the crude cells in a solution of Nycodenz® of density 1.077 g/ml to allow the DCs to float to the top during centrifugation, while all of the denser cells pellet (e.g. refs 6-9). This strategy too has been adapted to OptiPrep™, but to improve the purity of the DCs there has been a tendency to reduce the density of the suspending solution (approx 1.061 g/ml). This separation protocol was first described for the isolation of DC from mouse Peyer's patches, lymph nodes and spleen and for the isolation of Langerhans cells from skin [10-12]. The method is described in this Application Sheet and it is the easiest of all the methods to execute.

A third option, also a flotation technique is as follows: the cell suspension is adjusted to a density of 1.085 g/ml with OptiPrep™and the DC allowed to float up through an iodixanol solution of density 1.065 g/ml layered on top. This is described in **"Dendritic cells" Application Sheet C21, see index.**

2. Solutions required (see Note 1 for the Nycodenz® option)

Keep the following stock solutions at 4°C:
100 mM Tricine: 1.79 g per 100 ml water; 100 mM EDTA ($Na_2.2H_2O$): 3.72g per 100 ml water

Solution D: Dissolve 0.8 g NaCl in 50 ml water; add 10 ml and 5 ml respectively of stock buffer and EDTA solutions; adjust to pH 7.4 with NaOH and make up to 100 ml.

A. OptiPrep™ (shake the bottle gently before use)
B. Digest solution: RPMI containing 5% heat-inactivated fetal calf serum (FCS), 10 U/ml collagenase and 5 μg/ml DNase I, pH 7.4 (see Note 2).
C. Phosphate buffered saline (without Ca^{2+} and Mg^{2+}) containing 5% fetal calf serum (FCS) 5 mM EDTA and 5 μg/ml DNase I.
D. Diluent: 0.8% (w/v) NaCl, 5 mM EDTA, 10 mM Tricine-NaOH, pH 7.4
E. 30% (w/v) Iodixanol working solution: mix equal vols. of OptiPrep™ and Solution D.

3. Protocol (adapted from refs 3 and 4)

3a. Dissociation of the tissue (see Note 3)

Preparation of a single cell suspension by dissociation of the chosen tissue will only be described in general terms in this Application Sheet. Detailed protocols may vary from laboratory to laboratory.

1. Digest the finely chopped tissue in Solution B at 37°C for 10 min.

2. Pass the digest through a stainless steel sieve.

3b. Gradient separation

Carry out all operations at 4°C, making sure that all solutions and equipment are pre-cooled.

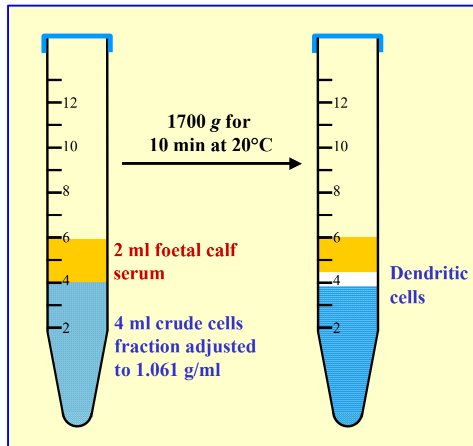

Figure 1: Purification of dendritic cells from lymphoid tissue by flotation (mixer technique)

1. Harvest the cells by centrifugation at 540 *g* for 5 min and wash them twice in Solution C.

2. Prepare an isoosmotic solution of 10.5% (w/v) iodixanol (ρ = 1.061 g/ml) by diluting 10.5 vol. of Solution E with 19.5 vol. of Solution D (see Notes 4-6).

3. Suspend the washed cell pellet in this solution at approx. 1.5×10^8 cells/ml.

4. Transfer 3-4 ml to a centrifuge tube and overlay with 2 ml of FCS (see Note 7).

5. Centrifuge at 1700 *g* for 10 min using a slow acceleration mode (see Note 8)

6. Allow the rotor to decelerate without the brake and harvest the DC from the FCS/sample interface (see Figure 1 and Note 9).

4. Notes

1. The most widely used Nycodenz® option was published in 1996 [6]. Prepare a stock solution of 1.16 g/ml as follows: place 50 ml of water or the chosen buffer in a 150 ml beaker on a heated magnetic stirrer set at approx. 50°C and add 30.5 g of Nycodenz® powder in small amounts until dissolved. Allow the solution to cool to room temperature and then make up to 100 ml with water or the buffer. Filter sterilize if required. To make a 1.077 g/ml solution dilute the stock to 14% (w/v) Nycodenz® with 0.154 M NaCl, 4 mM KCl, 5 mM EDTA, 5 mM HEPES-NaOH, pH 7.2 containing 5% FCS (adjusted to 10 mM EDTA).
2. The composition of the digest medium is only given in outline; other components such as antibiotics or glutamine may be included as required by the operator.
3. The operator should use whatever digest protocol is effective for the chosen tissue (see refs. 1 and 2 for more information). It is important however that the handling of the cells after digestion should be carried out as gently as possible to avoid potential damage to the cells. DNase I and/or EDTA are included in the cell medium to reduce any cell aggregation to a minimum.
4. The composition of the isoosmotic solutions used in the gradient separation may be adjusted to suit the requirements of the operator. EDTA prevents any aggregation.
5. For the Nycodenz® option, the cells should be suspended in the 14% (w/v) Nycodenz® solution (see Note 1). The density of the solution used to suspend the cells should be adjusted to suit the operator's requirements. Iodixanol or Nycodenz® solutions up to 14.5% (w/v) may be used.
6. The density of the DC may depend on the pre-gradient treatment and also the material source. It may be necessary to modulate the density of the cell suspension medium in the light of experience.

7. It is important to put a low-density layer on top of the cell suspension, to avoid banding the DC at an air/water interface. This low-density layer however could alternatively be Solution D (± FCS).
8. Centrifugation conditions vary quite widely; times as long as 20 min at 1,700 *g* [13] or *g*-forces as low as 600 *g* for 25 min [14,15].
9. The harvested DC may be further purified using the appropriate MAb-coated magnetic beads. This initial gradient purification step allows the bead purification to be performed more efficiently.

5. References

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