

OptiPrep™ Application Sheet C41

Isolation of dendritic cells from tissues by sedimentation on to a density barrier

- ◆ **OptiPrep™** is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- ◆ The **OptiPrep™ Reference List “Dendritic cells from blood and tissues” (RC05)** compares all of the currently available methodologies and provides a comprehensive bibliography of all the published papers reporting the use of OptiPrep™ listed according to the research topic: 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 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.

Nycodenz® density barriers of $\rho = 1.076$ g/ml [3] to 1.084 g/ml [4], the majority being approx 1.077 g/ml (e.g. refs. 5-9), have been used in the “traditional” format in which the crude cell fraction is layered on top. There are also instances of the use of the customized medium Nycoprep™ 1.068 [10,11], which was designed for the purification of monocytes from a human leukocyte-rich plasma. This strategy of layering the cell suspension on top of a barrier has been extended to the use of OptiPrep™ for isolation of DCs from lymph [12], bone marrow [13], bone marrow cell cultures [14], spleen [15], thymus [16] and lung [17].

- ◆ For alternative flotation protocols see **Application Sheets C21 and C22**

2. Preparation of cells

For the standard preparation of DCs from spleen and thymus by collagenase digestion and filtration see **Application Sheet C21**. Lung DCs are prepared by perfusion of the tissue via the pulmonary artery with phosphate-buffered saline containing heparin prior to digestion with collagenase (see ref. 15 for more details). For the preparation of DCs from bone marrow cell cultures, the cells flushed from murine femurs and tibias are cultured in the standard RPMI/10% FCS in the presence of 10 ng/ml IL-4 and granulocyte-macrophage colony-stimulating factor for 6 days prior to the harvesting of loosely adherent and non-adherent cells (see ref. 14 or more details of this cell preparation protocol).

3. Purification of dendritic cells (adapted from ref 14)

3a. Solutions required

- A. OptiPrep™ (shake the bottle gently before use)
- B. Diluent: 0.88% (w/v) NaCl, 1 mM EDTA, 0.5% bovine serum albumin (BSA), 10 mM Tricine-NaOH, pH 7.4 (see Note 1)
- C. Density barrier: 2.3 vol. of Solution A + 9.7 vol. of Solution B (see Note 2)
- D. Hank’s Balanced Salt Solution (see Note 3)

Keep the following stock solutions at 4°C:
 100 mM Tricine: 1.79 g per 100 ml water
 100 mM EDTA (Na₂·2H₂O): 3.72g per 100 ml water

Solution B: Dissolve 0.88 g NaCl in 50 ml water; add 10 ml and 1 ml respectively of stock buffer and EDTA solutions; adjust to pH 7.4 with NaOH; dissolve 0.5 g of bovine serum albumin in the solution; check the pH and make up to 100 ml.

3b. Protocol

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

1. Harvest the cells by centrifugation at 540 g for 5 min and wash them twice in Solution D.
2. Suspend the washed cell pellet in this solution (6 ml).
3. Transfer 6 ml of Solution C or other chosen density barrier to a centrifuge tube and overlay with cell suspension (see Note 4).
4. Centrifuge at 600-700 g for 5-10 min; use a slow acceleration if available (see Note 5).
5. Allow the rotor to decelerate without the brake and harvest the DCs from the interface (see Note 6).

4. Notes

1. In some cases the OptiPrep diluent is a culture medium supplemented with FCS and EDTA. Any approximately isoosmotic solution compatible with the cells may be used.
2. The density of this barrier is approx. 1.067 g/ml and equivalent to 11.6% (w/v) iodixanol; others have used 12% [15]. For lung DCs Hansen et al [17] employed a two-layer gradient of 4% and 16% (w/v) iodixanol.
3. The solution used to suspend the cells prior to layering on the density barrier and the solution used to dilute the OptiPrep™ may be any compatible isoosmotic medium. Hansen et al [17] used Hank's Balanced Salt Solution (HBSS) containing 5% FCS and 2 mM EDTA for both, while Cervantes-Barragan et al [15] replaced the HBSS with PBS and Luckashenak et al [16] used PBS for suspending the cells and HBSS for diluting the OptiPrep™ (both containing 0.2% BSA and 2 mM EDTA).
4. The relative volumes of sample and density barrier are probably not very critical; 2ml over 2 ml is a quite common format with Nycodenz® barriers.
5. The time may be increased to 20 min. Most centrifugations are carried out at 4°C but higher temperatures may be used. There is a broad range of centrifugation conditions with Nycodenz® barriers; approx. 800 g for 20 min is common but 530 g for 20 min to 2800 g for 15 min have been described.
6. In the case of the two-layer gradient, the DCs band at the lower interface. Eaton et al [14] reported that the purity of the DCs from bone marrow cultures, as judged by flow cytometry, was approx. 85%. The harvested DC may be further purified by negative selection using the appropriate MAb-coated magnetic beads. Carrying out this initial gradient purification step allows the bead purification by negative selection to be performed more efficiently. Ref. 19 reviews DC methodology and function.

5. References

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