# OptiPrep™ Application Sheet C21

## Dendritic cells from tissues by flotation through a low-density barrier

- 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 list 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 in index.**

Another common approach is to suspend the crude cells in a solution of Nycodenz® of density 1.077 g/ml and then allow the DCs to float to the top during centrifugation (e.g. refs 6-9). This approach 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 (as low as 1.061 g/ml). It is described in **"Dendritic cells" Application Sheet C22 in index.**

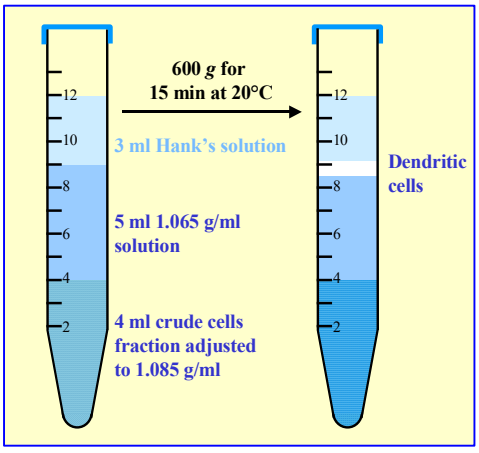


**Figure 1:** Purification of dendritic cells by flotation through a low-density barrier from a high-density cell suspension (for details see text)

The protocol described in this Application Sheet was first described for the isolation of DC from mouse Peyer's patches [10], but it has since been extended to their isolation from blood, lymph nodes, spleen and thymus and the isolation of Langerhans cells from skin. Like that in **Application Sheet C22** it involves flotation but the crude suspension is adjusted to 1.085 g/ml and the separating low-density barrier is layered on top (see Figure 1). The major advantage of this approach is that the DCs are separated from the original dense cell suspension by the low density barrier, which is free of other cells, any cells partially disrupted by the earlier disaggregation process or any residual enzymes used in this process. This is not the case with any other strategy. The 1.065 g/ml barrier almost acts as a "washing" solution. The following protocol is adapted from ref 10.

♦ Note that in many cases the density barrier enrichment of DCs is followed by further purification using antibody-bound beads

**2. Solutions required**

**A.** OptiPrep™ (shake the bottle gently before use)

**B.** Suspension solution: Hank's Balanced Salt Solution (without $Ca^{2+}$ and $Mg^{2+}$)

**C.** Diluent: 0.88% (w/v) NaCl, 1 mM EDTA, 0.5% (w/v) bovine serum albumin (BSA), 10 mM HEPES-NaOH, pH 7.4.

Keep the following stock solutions at 4°C;
100 mM HEPES (free acid) 2.38 g per 100 ml water
100 mM EDTA ($Na_2$•$2H_2O$) 3.72 g per 100 ml water

Solution C: Dissolve 0.88 g NaCl and 0.5 g BSA in 50 ml water; add 10 ml and 1 ml respectively of stock HEPES and EDTA solutions; adjust to pH 7.4 with NaOH and make up to 100 ml.

**D.** Digest solution: RPMI (DMEM or IMDM) containing 5% fetal calf serum (FCS), 10 U/ml collagenase (see Note 1).

**3. Protocol**

**3a. Dissociation of the tissue** (see Note 2)

1. 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.

2. Digest the finely chopped tissue twice in the Solution D at 37°C for 30 min.

3. Pass the digest through a stainless steel sieve.

4. Harvest the cells by centrifugation and wash them as required in a balanced salt medium (see Note 3).

**3b. Gradient separation** (see Note 4)

1. Make up an 11.5% (w/v) iodixanol solution (ρ = 1.065 g/ml) from Solutions A and C (1:4.2 v/v).

2. Suspend the washed cell pellet in Solution B and mix gently but thoroughly with OptiPrep™ (3:1 v/v) to give a 15% (w/v) iodixanol solution (ρ = 1.085 g/ml). Overlayer 4 ml of this suspension with 5 ml of the 11.5% iodixanol solution (step 1) and 3 ml of Solution B.

3. Centrifuge at 600 $g_{av}$ for 15 min at room temperature (approx 20°C).

4. Allow the rotor to decelerate without the brake and harvest the DC from the top of the 11.5% iodixanol layer (see Figure 1 and Note 5).

**4. Notes**

1. The composition of the digest medium is only given as a basic recipe, other components such as antibiotics; glutamine may be included as required by the operator.
2. The operator should use whatever digest protocol is effective for the chosen tissue. 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. Sometimes DNase I and/or EDTA are included in the final cell suspension medium to reduce any cell aggregation.
3. This medium should not contain $Ca^{2+}$ or $Mg^{2+}$ but inclusion of FCS may be permissible.
4. Some publications report the use of an upper layer of density higher than 1.065 g/ml (up to 1.071 g/ml) and media of a composition different to that of Solution C for diluting the OptiPrep™. More information on gradient solution preparation is in **Application Sheet C01**.
5. Ruedl et al [10] reported that from Peyer's patch material 3-5% of the total cells in the gradient were recovered at the upper interface, and the enrichment of DC over the starting material should be 30-60x (Ruedl, C. *personal communication)*.

## 5. References


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## 6. Acknowledgements

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