

OptiPrep™ Application Sheet C45

Purification of macrophages and foam cells

- ◆ OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- ◆ 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. Introduction

This Application Sheet is concerned primarily with the purification of macrophages from either alveolar lavages or peritoneal fluid (Section 2); however these cells have also been isolated from a variety of other sources such as lung tissue, intestinal lamina propria and spinal cord tissue (see Section 3). Section 4 is concerned with the foam cells found in atherosclerotic lesions. Section 5 describes more recent applications using OptiPrep™.

It is also recognized that macrophages are related to dendritic cells from a variety of tissue sources and to blood monocytes. For the purification of these cells please refer to the following Application Sheets:

- ◆ Dendritic cells – barrier flotation: **Application Sheet C20**
- ◆ Dendritic cells – barrier sedimentation: **Application Sheet C46**
- ◆ Dendritic cells – mixer flotation: **Application Sheet C21**
- ◆ Monocytes – Leukocyte-rich plasma – barrier sedimentation: **Application Sheet C51**
- ◆ Monocytes – Leukocyte-rich plasma – flotation: **Application Sheet C09**
- ◆ Monocytes – whole blood, from: **Application Sheet C10**

2. Isolation from peritoneal fluid and alveolar lavages

There is no single methodology that has been widely adopted for the purification of macrophages, so this Application Sheet provides a selection of density gradients, taken from the literature. Many of the published papers report the use of Nycoprep™ 1.15, an isoosmotic solution of 27.6% (w/v) Nycodenz® in 3 mM KCl, 0.3 mM CaNa₂-EDTA 5 mM Tris-HCl, pH 7.5. This is no longer commercially available, so we suggest that solutions of the same density and osmolality should be prepared from OptiPrep™. The alternative in many cases is to prepare the solutions from Nycodenz® powder; this is time consuming and the solutions will require sterilization. See **Application Sheet C01** for more information on preparing gradient solutions from OptiPrep™.

2a. Sedimentation on to a density barrier

2a-1. Background

Rat peritoneal macrophages require separation from lymphocytes, neutrophils and erythrocytes; Fisker et al [1] carried out a detailed study of the gradient requirements for unstimulated rats and for rats receiving thioglycollate. The optimal properties of the density barrier that produced the highest yields and purity of macrophages were different for the two types of animal. For the normal animal the optimal density and osmolality were 1.091 g/ml and 325 mOsm; for the stimulated rat the figures were 1.106 g/ml and 400 mOsm. Data in subsequent papers confirmed this [2,3]. Fisker et al [1] used Nycoprep™ 1.15; since this solution is isoosmotic it is simple to mimic this solution with OptiPrep™. The following method is adapted from ref 1.

2a-2. Solutions required (see Box on next page for more details about solution preparation)

- OptiPrep™ (shake the bottle gently before use)
- OptiPrep™ diluent: 0.85% (w/v) NaCl, 6 mM KCl, 0.6 mM EDTA, 0.6 mM CaCl₂, 10 mM HEPES-NaOH, pH 7.0
- Hyperosmotic mixer: 1.245 M NaCl, 3 mM KCl, 0.3 mM EDTA, 0.3 mM CaCl₂, 5 mM HEPES-NaOH, pH 7.0

2a-3. Protocol

1. Prepare the 27.6% (w/v) iodixanol stock solution by mixing 27.5 vol. of Solution A with 32.5 vol. of Solution B.
2. Select the appropriate density barrier (see Section 2a-1); for the 1.091 g/ml/325 mOsm barrier, mix 0.6 vol. of the 27.5% (w/v) iodixanol stock solution with 0.34 vol. of water and 0.06 vol. of Solution C; 1.106 g/ml and 400 mOsm barrier, mix 0.7 vol. of the 27.5% (w/v) iodixanol stock solution with 0.22 vol. of water and 0.08 vol. of Solution C (see Note 1).
3. If possible check the osmotic pressure with an osmometer.
4. Layer 7 ml of the cell suspension over 3 ml of the chosen density barrier and centrifuge at 700 g for 20 min at room temperature.
5. Collect the macrophages from the interface.

Keep the following stock solutions at 4°C
 100 mM HEPES (free acid): 2.38 g per 100 ml water;
 adjust to pH 7.0 with 1 M NaOH before making up to final volume.

100 mM EDTA (Na₂•2H₂O): 3.72 g per 100 ml water
 1 M M KCl: 7.46 g per 100 ml water
 100 mM CaCl₂•2H₂O: 1.47 g per 100 ml water

Solution B: Dissolve 0.85 g NaCl in 50 ml of water
 add 10 ml of the HEPES stock solution and 0.6 ml of each of the other stock solutions; adjust to pH 7.0 and make up to 100 ml with water

Solution C: Dissolve 7.28 g NaCl in 50 ml of water;
 add 5 ml of the HEPES stock solution and 0.3 ml of each of the other stock solutions; adjust to pH 7.0 and make up to 100 ml with water

2a-4. Notes

1. The only other example of a density barrier was very different to that described above. Cells from a peritoneal cavity lavage in RPMI were layered on NycoPrep 1.068™ (600 g for 10 min). This commercial medium is also no longer available and its primary purpose was to isolate human monocytes from a leukocyte-rich plasma. The macrophages are described as banding in a median zone within the NycoPrep 1.068™ [4]. Like the density barriers described above it does have a raised osmolality. A medium of identical density and osmolality may again be readily prepared from OptiPrep™ and this is described in [Application Sheet C51](#).

2b. Two-layer discontinuous gradients**2b-1. Flotation**

In the first example of a two-layer gradient for peritoneal macrophages [5], a stock solution of approx. 25% (w/v) Nycodenz®, 10 mM EDTA was diluted with Hank's Balanced Salt Solution (HBBS) (containing 10 mM EDTA) to produce two solutions of approx. 19% and 14.5% Nycodenz®. The cells from the peritoneal lavage were suspended in the 19% Nycodenz® (12ml); the 14.5% (16 ml) Nycodenz® and a layer of HBBS (12 ml) were laid on top. After 30 min at 400 g the macrophages banded above the 14.5% layer and the neutrophils below it. The density solutions can be made up from OptiPrep™: dilute with an equal volume of HBSS containing 20 mM EDTA to produce a 30% (w/v) iodixanol solution; then dilute further to 14.5% and 19% (w/v) iodixanol with HBSS-10 mM EDTA.

2b-2. Sedimentation

The method developed by Freeman et al [6] for the separation of neutrophils and mononuclear cells from blood has also been used for the isolation of rat alveolar macrophages [7]. This methodology is described in [Section 3 of Application Sheet C44](#).

2c. Multi-step gradients

Separation of alveolar and peritoneal macrophages from lymphocytes and neutrophils has occasionally been executed in four-step discontinuous gradients, the density solutions being prepared by dilution of NycoPrep™ 1.15 with PBS containing 4 mM EDTA [8]. The gradient of approx. 12%, 14.5%, 17.5% and 20% (w/v) Nycodenz® was centrifuged at 500 g for 45 min and macrophages banded at the interface of the top two layers. To produce these density solutions from OptiPrep™ and

to mimic the EDTA profile of the Nycodenz® gradient, first dilute OptiPrep™ with an equal volume of PBS to produce a 30% (w/v) iodixanol solution and then dilute further with PBS containing 4 mM EDTA to make solutions of 12%, 14.5%, 17.5% and 20% (w/v) iodixanol.

3. Isolation from spinal tissue and lamina propria

Beck et al [9] described a four-layer discontinuous gradient of density 1.029, 1.037, 1.056 and 1.061 g/ml. The solutions were produced by dilution of OptiPrep™ with 0.15 M NaCl, 10 mM MOPS, pH 7.4. 1 ml of each of the solutions was overlaid by 6 ml of the crude spinal cord cells and centrifuged at approx 700-800 g for 15 min at room temperature. The glial and inflammatory cells were recovered from the pellet. Iodixanol gradients have also been used for spinal cord tissue by Galvan et al [10] and Mavrikis Cox [11].

Lamina propria macrophages were suspended in approx. 5 ml of 5.5% (w/v) Nycodenz® and layered over 3 ml 11% (w/v) Nycodenz®. After centrifugation at 650 g for 20 min the macrophages banded at the interface [12]. The solutions were prepared by dilution of NycoPrep™ 1.15 with PBS; solutions of approx the same density (5.5% and 11% w/v iodixanol) may be prepared by simple dilution of OptiPrep™ with PBS.

4. Foam cells

Foam cells, being lipid-laden macrophages from atherosclerotic lesions are likely to be less dense than other macrophages. After collagen treatment of the lesion-containing material and sieving, the washed cells were layered over a five-layer discontinuous Nycodenz® gradient of 0.5, 1.0, 5.0, 10 and 30% (w/v) Nycodenz® and centrifuged at 1200 g for 15 min at 10°C [13,14]. The foam cells banded at the 5%-10% interface. A gradient of exactly the same density profile can be generated by diluting OptiPrep™ with buffered saline, balanced salt solution or culture medium to give the same % (w/v) iodixanol solutions.

5. Publications reporting the use of OptiPrep™

Macrophages and hepatic stellate cells have been separated from liver by successive centrifugations on 8.2% and 17.6% (w/v) iodixanol barriers [15]. Sometimes the crude liver cell fraction is adjusted to 17% (w/v) iodixanol, overlaid with a buffered salt solution; after centrifugation the macrophages have floated to the interface [16]. Hepatic macrophages have also been recovered on a much lower density barrier of 8.2% (w/v) iodixanol [17]. Other papers also report the use of iodixanol gradients tissues for liver macrophages (e.g. refs 18 and 19).

Macrophages from other sources that have been isolated in iodixanol gradients are: human tumour tissue [20], lung tissue [21,22], mouse small intestine [23] and rabbit peripheral blood [24]. Macrophages have also been recovered from subcutaneous surgical sponge implants in mice by flotation on a 10% (w/v) iodixanol barrier [25].

6. References

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