

OptiPrep™ Application Sheet C40

Isolation of mononuclear cells from tissues

- ◆ OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- ◆ **Axis-Shield Mini-Review (MC01)** “Purification of mononuclear cells, monocytes and polymorphonuclear leukocytes – a methodological review” compares all of the currently available methodologies
- ◆ **Axis-Shield Mini-Review (MC02)** “Purification of mononuclear cells, monocytes and polymorphonuclear leukocytes – a bibliographical review” provides a comprehensive bibliography of all the published papers reporting the use of OptiPrep™
- ◆ To access **MC01 and MC02** return to the initial list of Folders and select “**Mini-Reviews**”.
- ◆ 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

Mononuclear cells (MCs) from a variety of tissues, predominantly liver, spleen, intestine and bone marrow, have been purified by using a strategy similar to that used for the isolation of these cells from blood, namely sedimentation onto a density barrier. This density barrier has commonly been a Nycodenz® solution, usually in the form of one of the ready-made Nycoprep™ solutions; sometimes Nycoprep™ 1.077 [1,2], but much more frequently Nycoprep™ 1.077A [3-8]. The former was originally formulated for the isolation of MC from human blood, the latter from rodent blood. Nycoprep™ 1.077A is no longer commercially available but a solution of the same density and osmolality may very easily, be prepared from OptiPrep™ (see Section 3).

More recently MCs from liver have been purified on an iodixanol density barrier of approx $\rho = 1.084$ g/ml [9,10] or banded between a two-layer gradient of 1.051 and 1.077 g/ml [11]. Other tissues from which MCs have been prepared using sedimentation onto an iodixanol density barrier are rat spleen [12], spinal cord [12] and bone marrow [13-15]. As far as is known, these barrier sedimentation methods have not been executed using a low-osmolality density barrier. The iodixanol barrier solutions have been produced by dilution of OptiPrep™ with regular saline or culture medium.

In the alternative “mixer” strategy the sample is simply adjusted to a density just higher than that of the MCs so that the latter float to the top during the centrifugation, introduced for human blood by Ford and Rickwood [16] using Nycodenz®. This was later adapted to the use of OptiPrep™ and extended to both mouse and rat blood. It has now been used successfully for the isolation of MCs from liver [17-22] and spleen [23,24]. This technology should be applicable to any mouse or rat tissue.

The most commonly used tissues for the isolation of lymphocytes are bone marrow, spleen, intestine and liver. Since this Application Sheet was first prepared however, the use of iodixanol gradients has additionally been reported for their isolation from brain, heart and lung tissue. Although the general gradient strategies have not changed, there is considerable variation in some of the details, some of which will be indicated in Section 6.

- ◆ This Application Sheet presents the density gradient methods for resolving the lymphocytes and not the methods that are used to disaggregate the tissues. **Methods for preparing a total non-parenchymal cell fraction are described in Application Sheet C24**
- ◆ Section 2 describes the options for sedimentation on to a density barrier
- ◆ Section 3 describes the two-layer gradient
- ◆ Section 4 describes the flotation strategy
- ◆ Section 5 contains important notes to Sections 2, 3 and 4
- ◆ Section 6 briefly describes some of the methodological variations reported in recent papers

2. Sedimentation on to a density barrier

2a. Solution preparation

- A. OptiPrep™ (60%, w/v iodixanol) – shake the bottle gently before use
- B. Buffered saline

Keep Hepes (free acid) or Tricine as a 100 mM stock solution at 4°C; Hepes (2.38 g) or Tricine (1.79 g) per 100 ml water.

Solution B: Dissolve 0.85 g of NaCl in 50 ml water; add 10 ml of Hepes or Tricine stock solution; adjust to pH 7.2-7.4 with 1 M NaOH and make up to 100 ml.

2a-1 Isoosmotic barrier solution

Dilute Solution A with Solution B to obtain isoosmotic solutions of lower density. The density of this barrier solution may be modulated to improve either the purity or yield of mononuclear cells. For more information on the density of iodixanol solutions see [Application Sheet C01](#). The published methods quote % (w/v) iodixanol concentrations from 12.6% (see ref 17), which has a density of approx. 1.072 g/ml, to 15% (e.g. see refs 9, 10, 25) with a density of approx. 1.084 g/ml and also includes the standard 1.077 g/ml barrier [26] and for intestinal cells a much lower density barrier (1.055 g/ml) was employed at 1700 g for 10 min [27]. Centrifugation conditions are normally in the range 600-1000 g for 10-20 min.

2a-2 Hypoosmotic 1.077 g/ml barrier solution

Dilute Solution B with water (2.5 vol. + 0.5 vol.) and then mix 2.7 vol. of Solution A with 9.3 vol. of the diluted saline solution (see Note 1).

2b. Protocol (adapted from refs 9 and 10)

1. Layer 2 vol. of cell suspension (see Section 2) on top of 1 vol. of the density barrier (see Note 2).
2. Centrifuge at 750 g for 20 min at room temperature (see Note 3).
3. Allow the rotor to decelerate without the brake and harvest the MCs from the interface.

3. Sedimentation in a two-layer gradient

3a. Solutions required

- A. OptiPrep™ (60%, w/v iodixanol)
- B. Culture medium (RPMI 1640)

3b. Protocol (adapted from ref 11)

1. Shake the OptiPrep™ gently before use.
2. Make up two solutions of 1.051 and 1.078 g/ml by mixing Solutions A and B in the following volume ratios (1:5.8) and (1:3.5) respectively (see Note 4)
3. Suspend the cells in the 1.051 g/ml solution and layer over an equal volume of the 1.078 g/ml solution (see Note 2).
4. Centrifuge at 750 g for 20 min at room temperature.
5. Allow the rotor to decelerate without the brake and harvest the MCs from the interface (see Note 5).

4. Flotation strategy

4a. Solutions required

- A. OptiPrep™ (60%, w/v iodixanol)
- B. Culture medium (RPMI 1640)

4b. Protocol (adapted from ref 20)

1. Shake the OptiPrep™ gently before use and mix 4 vol. of Solution A with 2 vol. of Solution B to produce a solution of density 1.215 g/ml (see Note 7).

2. Suspend the cells in 3.9 ml of Solution B and mix gently but thoroughly with 2.1 ml of the 1.215 g/ml solution (see Note 8).
3. Layer 1 ml of Solution B on top and centrifuge at 1500 g for 20 min at 4°C (see Notes 5 and 9).
4. Allow the rotor to decelerate without the brake and harvest the MCs from the interface.

5. Notes

1. This solution is equivalent to Nycoprep™ 1.077A and has a density of 1.077 g/ml and an osmolality of approx 265 mOsm.
2. The relative volumes of sample and density barrier are probably not critical but the given ratio is widely used.
3. The separation on an isoosmotic barrier should not be temperature dependent but lower temperatures may require a further 5 min of centrifugation because of the increased viscosity at lower temperatures. Use of the hypoosmotic medium should be carried out at room temperature because the movement of water across an osmotic gradient is reduced at low temperatures.
4. The density of the 1.078 g/ml solution might be modulated upwards if too many MCs are lost to the pellet. For intestine and liver mononuclear cells Zellweger et al used densities of 1.052 and 1.076 g/ml [28]. This was also very similar to the method of Dai et al [29]
5. The separation should not be temperature dependent but lower temperatures may require a further 5 min of centrifugation because of the increased viscosity at lower temperatures.
6. The ratio of Solution A:Solution B may be modulated in the light of data on the recovery of MCs. In step 3 it is possible to mix the sample directly with OptiPrep™ rather than with the $\rho = 1.215$ g/ml medium; the latter is the chosen method because complete mixing of OptiPrep™ with the sample requires rather more vigorous agitation, which may be deleterious to the cells.
7. The final v/v ratio of OptiPrep™ in the sample is 22%, variations include 21% [17,18], 21.5% [21,22] and 24% [23]. So the final concentration of iodixanol varies from 12.6% (w/v) to 14.4% (w/v), equivalent to densities of approx. 1.072-1.082 g/ml.
8. The small layer of culture medium on top of the sample does not influence the separation, but it prevents the MCs from banding at an air/liquid interface, which causes aggregation problems.
9. For bone marrow cells, 30 ml of cell suspension was mixed with 10.1 ml of OptiPrep™ and 4 ml of water; then overlaid with 5 ml of PBS and centrifuged at 1000 g for 30 min [30] – note that the final iodixanol concentration in the sample was 13.7% (w/v).

6. Recent methods and variations in methodology

- ◆ Lung lymphocytes have been purified on both the normal density barrier [31] and a slightly denser barrier of approx 16.5% (w/v) iodixanol (described in the paper as 27.5% (v/v) OptiPrep™) [32] similar to that used for intestinal cells [33].
- ◆ Two-layer gradients of 15% (w/v) and 11% (w/v) iodixanol (equivalent to 1.085 and 1.063 g/ml density) were applied to resolve the lymphocytes (in the top zone of the 15% layer and macrophages + dendritic cells just above the interface [34])
- ◆ Brain lymphocytes have only relatively recently been isolated using OptiPrep™ using a three-layer gradient of 5%, 10% and 18% (w/v) iodixanol at 800 g for 30 min; the leukocytes collected at the 10-18% interface [35-37]

7. References

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