C40 Isolation of mononuclear cells from tissues

- **OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml**
- For links to other relevant files click on the double blue arrow in the following text

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 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.

- Note that Nycoprep™ 1.077A however is no longer commercially available from Axis-Shield but it is easy to prepare a density barrier solution from OptiPrep™ of the same density and osmolality as Nycoprep™ 1.077A.

More recently MCs from liver have been have been purified on an iodixanol density barrier of approx ρ = 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].

The alternative "mixer" strategy for the isolation of MCs in which 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 was first 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 very successfully for the isolation of MCs from liver [17-22] and spleen [23]. It is unlikely that the density of MCs is tissue specific and so this technology should be applicable to any mouse or rat tissue.

The following protocols describe the isolation of mononuclear cells from liver.

- Section 2 gives a brief summary of the methods for disaggregating the liver with collagenase and preparation of the cell suspension
- Section 3 describes the options for sedimentation on to a density barrier
- Section 4 describes the two-layer gradient
- Section 5 describes the flotation strategy

2. Disaggregation of tissue and preparation of the cell suspension

There are many variations in technique for the production of a single cell suspension. In many cases, after cutting the inferior vena cava, the liver is perfused via the portal vein with 50 U/ml of collagenase IV; removed from the body cavity; passed through a metal sieve or cell strainer and then further incubated with 50U/ml collagenase IV in RPMI containing 5% fetal calf serum at 37°C for 30 or 40 min [9,10]. The pore size of the sieve or strainer is usually approx 70 μm. The initial liver perfusion may simply use a buffered saline to remove the blood, restricting the collagenase exposure to the sieved tissue material [20]. Lian et al [11] considered a number of variants to this procedure including dicing of the liver after the first perfusion with collagenase and passage through nylon mesh after a collagenase digestion for 20 min.

The preponderant parenchymal cells are removed by low speed centrifugation at 75 *g* for 1 min [11] or 30 *g* for 3 min [20]. The remaining non-parenchymal cells and leukocytes are then harvested at approx 120 *g* for 10 min [20]. They may be washed once to remove as much of the collagenase as possible before resuspension in culture medium or any other suitable medium.

3. Sedimentation on to a density barrier

3a. Solutions required

A. OptiPrep™ (60%, w/v iodixanol)

B. Culture medium (RPMI 1640) or any buffered saline (see box on right)

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

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

3b. Protocol (adapted from refs 9 and 10)

Two options for the density barrier are given below. Step 2 produces an isoosmotic solution [9,10], Step 3 produces a slightly hypoosmotic solution that mimics Nycoprep 1.077A.

1. Shake the OptiPrep™ gently before use.

2. Dilute Solution A with Solution B (1 vol. + 3 vol.) to produce a solution of 1.084 g/ml **OR**

3. Dilute the buffered saline solution with water (2.5 vol. + 0.5 vol.) and then mix 2.7 vol. Solution A with 9.3 vol. of the diluted saline solution (see Notes 1 and 2).

4. Layer 2 vol. of the cell suspension (see Section 2) on top of 1 vol. of the chosen density barrier (see Note 3).

5. Centrifuge at 750 *g* for 20 min at room temperature (see Note 4).

6. Allow the rotor to decelerate without the brake and harvest the MCs from the interface.

4. Sedimentation in a two-layer gradient

4a. Solutions required

A. OptiPrep™ (60%, w/v iodixanol)

B. Culture medium (RPMI 1640)

4b. 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 5)

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 3).

4. Centrifuge at 750 *g* for 20 min at room temperature (see Note 4).

5. Allow the rotor to decelerate without the brake and harvest the MCs from the interface.

5. Flotation strategy

5a. Solutions required

A. OptiPrep™ (60%, w/v iodixanol)

B. Culture medium (RPMI 1640)

5b. Protocol (adapted from ref 20)

1. Shake the OptiPrep™ gently before use.

2. Mix 4 vol. of Solution A with 2 vol. of Solution B to produce a solution of density 1.215 g/ml (see Note 2).

3. Suspend the cells in 1.8 ml of Solution B and mix gently but thoroughly with 2.2 ml of the 1.215 g/ml solution (see Note 6).

4. Layer 1 ml of Solution B on top and centrifuge at 1500 *g* for 20 min at 4°C (see Notes 4 and 7).

5. Allow the rotor to decelerate without the brake and harvest the MCs from the interface.

6. 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. For more information on preparing density solutions for cells see OptiPrep™ Application Sheet C1 ➔➔
3. The relative volumes of sample and density barrier are probably not critical but the given ratio is widely used.
4. The separation should not be temperature dependent but lower temperatures may require a further 5 min of centrifugation.
5. The density of the 1.078 g/ml solution might be modulated upwards if too many MCs are lost to the pellet.
6. The final v/v ratio of OptiPrep™ in the sample is 22%, variations include 21% [17,18] and 21.5% [21,22].
7. 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 viability and aggregation problems.

7. References

To access abstracts of refs 1-23 (file CA40) click on the double blue arrow ➔➔

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