

OptiPrep™ Application Sheet C35

Isolation of neuroglia, inflammatory and glial cells from neural tissue

- ◆ OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- ◆ The **OptiPrep™ Reference List RC06 “Isolation of neural cells from brain and spinal cord”** provides a comprehensive bibliography 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

In neural tissue there are numerous types of “support cells” which are grouped under the term “Neuroglia” and this Application Sheet is concerned with just two of them. Iodixanol gradients have been used to purify neuroglial cells and also to remove myelin from disaggregated CNS tissue prior to the use of antibody-bound beads to purify oligodendrocytes.

Microglial cells have been isolated both from mouse brain, using the standard methodology of tissue mincing followed by papain [1] or trypsin digestion [2] to disaggregate the cells. They have also been isolated from culture after a six-stage *in vitro* differentiation of mouse embryonic stem cells [2]. The latter involves expansion of undifferentiated stem cells, followed by generation of embryoid bodies, selection and expansion of nestin-positive cells, differentiation into neurons and expansion of microglia. Details of this methodology are beyond the scope of this Application Sheet and the reader is referred to ref 2 for details.

2. Purification of glial cells from mouse brain

For the gradient purification Bettinger et al [1] used four-step gradient which was overlaid by the sample, while Tsuchiya et al [2] used a two layer gradient flotation strategy; both are given in the protocol. More recently O’Mahony et al [3] also used a four-step gradient to resolve oligodendrocytes, neurons + glial accessory cells and microglia.

2a. Solutions required

- A. OptiPrep™ (shake the bottle gently before use)
- B. Hank’s Balanced Salt Solution (see Note 1)

2b. Protocol

1. Prepare the following iodixanol solutions by diluting OptiPrep™ with Solution B: for the four-step gradient [1] 4%, 5.5%, 7% and 10% (w/v) iodixanol **OR** for the two-step gradient [2] 9.6% and 21.6% (w/v) iodixanol (see Note 2).
2. Mince the brain tissue and pass through a 70 µm mesh sieve.
3. Suspend the mince in Solution B and dissociate the tissue by one of the following methods: (a) 500 µg papain per brain for 20 min at room temperature followed by DNase I (1 mg/ml) at 37°C for 5 min [1] **OR** (b) 0.25% trypsin and 0.1 mg/ml DNase I for 20 min at 37°C [2].
4. Dilute the suspension with Solution B and harvest the cells by centrifugation at 1,500 g for 10 min (see Note 3).
5. Resuspend the cell pellet in Solution B (for the four-step gradient) **OR** the 21.6% (w/v) iodixanol solution (for the two-step gradient).

6. For the four-step gradient, layer 1 ml of each of 4%, 5.5%, 7% and 10% (w/v) iodixanol and layer the cell suspension on top **OR** for the two step gradient layer an equal volume of 9.6% (w/v) iodixanol on top of the cell suspension in 21.6% (w/v) iodixanol (see Notes 4 and 5).
7. Centrifuge at 3000 g for 20 min (four-step gradient) **OR** 670 g for 20 min (two-step gradient) (see Notes 6 and 7).
8. In the four-step gradient the microglia band in the lowermost layer; in the two-step gradient, they band at the interface (see Notes 7-9).

2c. Notes

1. Any suitable medium – a buffered saline solution or a culture medium can be used in place of the Hank's Balanced Salt Solution.
2. The gradient used by O'Mahony et al [3] comprised 9%, 12%, 15% and 21% (w/v) iodixanol prepared by diluting OptiPrep™ with Hibernate A/B27 medium. While more recently Song et al [4] and Hong et al [5] pelleted the microglia through a 7.5%, 10.0%, 13.5% and 17% gradient (800 g for 15 min).
3. Tsuchiya et al [2] used a 5 min centrifugation for this step.
4. Larger gradient volumes may provide improved resolving power [3].
5. Discontinuous gradients are normally most easily prepared by underlayering (i.e. low density first) using a syringe (2 ml) and a long metal cannula; overlaying solutions, particularly those which differ in density by only a small amount, is more difficult. For more information about preparing gradients see [Application Sheet C02](#).
6. Tsuchiya et al [2] specified a temperature of 24°C for this step.
7. The gradient system described by O'Mahony et al [3] was centrifuged at 800 g for 15 min. After centrifugation the top 6 ml of the gradient was discarded as debris-containing, then in increasing density bands containing (1) oligodendrocytes, (2) neurons + glial accessory cells, (3) neurons and (4) microglia, were observed.
8. A 6% or 6.2% (w/v) iodixanol barrier has also been used in the separation of neurons and glial cells [6-10].
9. A barrier flotation method was employed by Tucsek et al [11]; cells were suspended in approx. 21% (w/v) iodixanol (OptiPrep™ diluted with Hank's balanced salt solution) and overlaid with 9.6% iodixanol. After 20 min at 670 g the microglia were harvested from the interface.

3. Removal of myelin for purification of cell fractions from rat brain

The trituration and incubation processes involved in the production of a suitable cell suspension for the isolation of oligodendrocytes from the mid-brain and cerebellar tissue from rat brain are very complex and may vary from laboratory to laboratory. For information on these methods see Section 4. This brief comment is concerned only with the simple one-step gradient strategy for removal of the myelin: Mix the dissociated cell suspension with OptiPrep™ so that the final iodixanol concentration is 9% (w/v) and centrifuge at 800 g for 20 min. Discard the myelin in the supernatant and harvest the cell-containing fraction in an appropriate medium (see refs 12-16).

4. Other neuronal cell purifications

Oligodendrocytes have been isolated on a four step-gradient prepared from OptiPrep™ with a purity of >90% [3,17-19]. The gradients are similar to those described in [“Isolation of brain motoneurons” Application Sheet C36 in index](#); the oligodendrocytes tend to be relatively low density and are recovered from the top of the gradient.

Astrocytes have also been purified in a four-step iodixanol gradient [20]. Firstly a working solution (WS) of approx 29.7% (w/v) iodixanol ($\rho = 1.161$ g/ml) by diluting OptiPrep™ with a 10 mM MOPS – 137 mM NaCl. This was further diluted with DMEM containing 10% foetal bovine serum (FBS). Complete culture medium (containing FBS) has a density of approx 1.009 g/ml. This was then used to dilute the iodixanol WS to produce the four solutions described in Table 1.

Table 1 Astrocyte-isolation gradient solutions

Density (g/ml)	Working Solution % (v/v)	Approx. % (w/v) iodixanol
1.076	35	10.4
1.062	25	7.4
1.056	20	5.9
1.049	15	4.5

A discontinuous gradient comprising 1 ml of each the gradient solutions was overlaid by 6 ml of the crude cell fraction and centrifuged at approx. 800 g for 15 min [20]. The astrocytes banded across the 1.062/1.056 g/ml interface. These cells have also been purified using the four-step iodixanol gradient described in **OptiPrep Application Sheet C36 (see above)** [21].

Iodixanol gradients are also a valuable aid to studies on the inflammatory response after traumatic spinal injury. By layering dissociated spinal cord cells over a four-step discontinuous gradient of 4.5%, 6%, 7.5% and 10.5% (w/v) iodixanol (OptiPrep™ initially diluted to 30% iodixanol with 0.15 M NaCl, 10 mM MOPS, pH 7.4 and then further diluted with Hanks Balanced Salt Solution) and centrifuging at 1900 rpm for 15 min., debris remained at the top interface, neurons banded at the three lower interfaces and inflammatory cells and glial cells pelleted [22,23]. Beck et al [24] compared OptiPrep™ with other methods including Percoll™ gradients and concluded that only OptiPrep™ gradients permitted a correct quantitative assessment of the presence of PMNs in spinal injury tissue.

◆ A methodological review of a number of OptiPrep™-based techniques is given in ref 25.

5. References

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