# OptiPrep™ Application Sheet C36

## Isolation of brain motoneurons

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- **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
- For the isolation of neuroglial cells **see OptiPrep™ Application Sheet C35**

### 1. Background

One of the first Nycodenz®-based methods for the isolation of hippocampal neurons from rat was published by Brewer [1]. It used a general-purpose commercial medium, NycoPrep™ 1.15 diluted with Hibernate A; the gradient comprised four layers of 15, 20, 25 and 35% (v/v) NycoPrep™ 1.15, equivalent to densities of approx 1.029, 1.036, 1.043 and 1.057 g/ml. The crude neuronal cell suspension (6 ml) was placed on top of 1 ml each of the gradient solutions. After centrifugation at 800 *g* for 15 min the disposition of cells was as shown diagrammatically in Figure 1. The sample zone contained mainly cellular debris; band A contained mainly oligodendroglia; neurons banded across the 1.043 g/ml layer and also within the 1.057 g/ml layer (B, C and D) and the pellet (E) contained mainly microglia [1]. The method was also applied to human post-mortem brain slices [2].

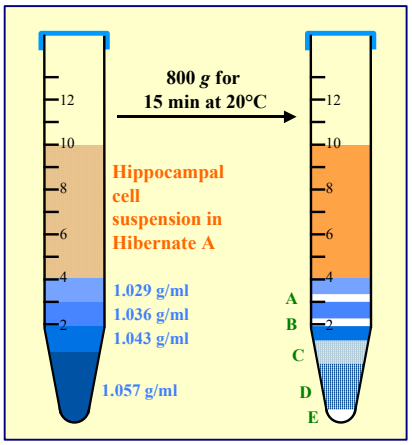


**Figure 1:** Purification of hippocampal neurons (see text for more information)

There are a couple of variants of the Nycodenz® gradient, both of which reduced the number of layers; one used three layers of 20, 35% and 100% (v/v) NycoPrep™ 1.15 [3] and the other used just 20% and 60% (v/v) NycoPrep™ 1.15 [4]. The ref 4 method also increased the *g*-force to 2000 *g* for 20 min. Note that NycoPrep™ 1.15 is no longer commercially available.

In 2001 Brewer et al [5] adapted Nycodenz® technology to OptiPrep™ and extended the method to human cortical tissue obtained at surgery; the density range of the gradient was again approx 1.029-1.057 g/ml. As with the Nycodenz® gradient (Figure 1) material is observed at each interface and in a pellet. Although neurons are present throughout the gradient, they are most highly enriched in fractions C and D [6]. Sometimes only the densest layer is harvested [5]. Marks et al [7] observed that the plating out of neurons directly from rat hippocampi after enzymic disaggregation and mechanical triturition became notably less efficient as the age of the animals increased from 8 to 35 days. However poor plating efficiency could be overcome by increasing the cell concentration in the suspension applied to substratum. This was achieved by banding the neurons in a four-step discontinuous iodixanol gradient covering the density range 1.026-1.055 g/ml. In this case the neurons were recovered from the lower layer after + the pellet after discarding the upper layers [7]. Liu et al [8] used a similar strategy, although the gradient density range was slightly different.

- The following protocol (adapted from refs 5 and 6) describes preparation of gradient solutions and the gradient centrifugation step, prior techniques for disaggregating the tissue are not included. An excellent detailed account of all the methodology associated with the isolation and culturing of neurons has been produced by Brewer and Torricelli [6].

**2. Solutions required**

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

**B.** OptiPrep™ diluent e.g. 0.85% NaCl, 10 mM MOPS-NaOH, pH 7.4 (see Note 1)

**C.** Working solution (30%, w/v) iodixanol): mix equal volumes of Solutions A and B.

**D.** Suspension medium (SM): see Note 2.

Keep MOPS (free acid) as a 100 mM stock solution at 4°C; 2.09 g per 100 ml water.

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

**3. Protocol**

1. Prepare a single cell suspension from the tissue by enyzmic disaggregation and triturition [8].

2. Suspend the cells in the medium of choice, this is normally Hibernate A (see Note 3) which can be supplemented as required [1-3].

3. Prepare gradient solutions of density 1.057, 1.043, 1.036 and 1.029 g/ml: mix Solution C with Solution D at these volume ratios: 0.33:0.67, 0.23:0.77, 0.18:0.82 and 0.13:0.87 (see Notes 4 and 5).

4. In a 15 ml centrifuge tube layer 1 ml of each of the four density solutions and then 6 ml of the cell suspension on top.

5. Centrifuge at 1900 rpm for 15 min at room temperature. Turn the brake off during the deceleration.

6. The distribution of material in the gradient is shown in Figure 1. Brewer et al [5] collected the fraction marked D, while Marks et al [7] included the pelleted material in the neuron harvest (see Note 6).

7. Dilute the fraction with 1-2 volumes of Solution D and harvest the cells by centrifugation.

**4. Notes**

1. The choice of OptiPrep™ diluent may vary with operational requirements. Brewer et al [3] observed that saline (0.8-0.9% NaCl) buffered with 10 mM MOPS-NaOH, pH 7.4 gave superior results. This diluent may however be any buffered isoosmotic solution; it may be, for example, Hibernate A (see Note 3). For more information about gradient solution preparation **see Application Sheet C01.**
2. Solution D is usually Hibernate A (see Note 3) but can be any suitable medium that is compatible with neurons. Ehrenreich et al [9] for example used Hanks Balanced Salt Solution; Vasko et al [10] used L-15 medium.
3. For sources of Hibernate A contact Dr Gregory Brewer, Dept of Molecular Biology, Microbiology and Biochemistry, South Illinois University School of Medicine, Springfield, IL 62794-9626 (gbrewer@siumed.edu).
4. The optimal density of the four layers may require some experimentation. For neurons from other species in particular, it may be necessary to modulate the density of the layers. Precise densities should be adjusted in the light of experience. More information on preparation of solutions of different densities can be obtained from **Application Sheet C01**.
5. The inclusion of a denser layer might be considered (e.g. 1.07 g/ml) if some denser contaminants are present. Whether improved resolution could be obtained by loading the sample in a dense solution below the gradient is one operational variant that might be considered.
6. The optimal harvesting procedure should be worked out once the composition of the zones of banded material (A-D in Figure 1) and the pellet has been verified.

## 5. References


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## 6. Acknowledgements

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