OptiPrep™ Application Sheet C23

Isolation of a mouse motoneuron enriched fraction from spinal cord on a density barrier

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- The **OptiPrep™ Reference List RC06** "Neural cells from brain and spinal cord – reference list" 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

The growth and differentiation of spinal motoneurons are dependent on various genetic and epigenetic factors, which influence both functional and morphological characteristics [1]. Involvement of a number of trophic molecules is known to be an important part of these processes [1]. Consequently there is a considerable amount of research carried out on cultured motoneurons, which can be derived from spinal cord.

The general procedure can be summarized as follows: Spinal cords are dissected from embryos and following a combined enzymic and mechanical disruption of the tissue, debris is removed by pelleting the cells through a cushion of bovine serum albumin. A motoneuron-rich fraction is then isolated from the cell pellet prior to cell culture.

Bataille et al [1] layered the crude cell pellet from rat embryo spinal cord over two layers of Nycodenz® of density, ρ = 1.047 and 1.065 g/ml and analyzed the cells that banded at the top of each Nycodenz® layer. The majority of the cells banded at the lower interface and the diameter of these cells (approx 4.3 μm) was much lower than the minor population of cells (motoneurons) around the upper interface (approx 6.7 μm). These large low-density cells contained very high levels of acetylcholine, which was virtually absent from the smaller denser cells at the lower interface. It seems clear from this earlier method that the motoneurons, as a result of their larger size are less dense than the other cells. Martinou [2] reported that the denser layer contained cholinergic cells other than motoneurons. In some cases the density of the two layers was changed, e.g. 1.035 and 1.092 g/ml [3] and for mouse embryo spinal cord 1.042 and 1.065 g/ml [4]. The gradient format was later simplified to a single low-density Nycodenz® cushion usually of density 1.055 g/ml [5,6], although densities as low as 1.035 g/ml have also been used [7].

Duong et al. [8] were first to report the use of OptiPrep™; they used a single layer of approx 10.5% (w/v) iodixanol (ρ = 1.06 g/ml) to purify mouse motoneurons, which remained on top of the density barrier after centrifugation. This simple barrier system has been described in all subsequent papers but, as with Nycodenz® methodology, the barrier density has often been reduced. For rodent motoneurons the iodixanol concentration may be 5.5-6.5% (w/v), equivalent to ρ = 1.035-1.040 g/ml [9-13] but for chick motoneurons [14,15] this as low as 5% (w/v) iodixanol (ρ = 1.035 = 1.032 g/ml).

Since the Nycodenz® and OptiPrep™ procedures are so similar, only the latter is given in this Application Sheet. It is based on ref 8.

2. Solutions required

A. OptiPrep™ (shake the bottle gently before use)
B. Hank's Balanced Salt Solution (without Ca^{2+} and Mg^{2+})
C. 3.5% (w/v) Bovine serum albumin in Solution B.
D. 0.025% trypsin in isotonic solution

3. Protocol

3a. Isolation of a total cellular fraction

1. Carry out all operations at room temperature

2. After dissection of the mouse embryo spinal columns, incubate them in Solution D for 20 min.

3. Dissociate the tissue by repeated passage through a syringe needle (21 gauge).

4. Layer the suspension over Solution C and centrifuge at 120 *g* for 10 min to remove cell debris.

5. Discard the supernatant and resuspend the pellet in Solution B.

3b. Isolation of a neuron-rich fraction

1. Dilute OptiPrep™ with Solution B to give a 1.06 g/ml solution, equivalent to 10.4% (w/v) iodixanol (see Notes 1 and 2).

2. Layer the resuspended pellet over the 1.06 g/ml solution.

3. Centrifuge at 400 *g* for 25 min (see Note 3).

4. Collect the banded cells in the upper layer, dilute with Solution B and centrifuge at 700 *g* for 10 min to pellet the motoneuron fraction.

5. Wash the cell pellet as required. See refs 1 and 7 for information on motoneuron culture (see Note 4).

4. Notes

1. For motoneurons from other species it may be necessary to modulate the density of the lower layer. For more information on gradient solution preparation see **Application Sheet C01**.
2. Modulating the density of the barrier will change the yield and purity of the motoneurons. Payne et al [16] isolated the largest cells from trypsinized mouse embryo spinal cords by sequential centrifugation over cushions of 5.2% iodixanol (15 min at 800 *g*) and 4% bovine serum albumin (10 min at 470 *g*). A similar strategy was used for chick embryo neurons [17].
3. The centrifugation conditions are quite varied, 800-900 *g* for approx 15 min is quite common [11,12,14,15], but *g*-forces as low as 100 *g* [13] have been used.
4. Misgeld et al [18] noted that from 1-2 mouse spinal cords, 10-25,000 motoneurons could be purified by this method.

5. References

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