OptiPrep™ Application Sheet C47

Purification of enterochromaffin-like (ECL) cells from gastric mucosa

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- 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
- Purification of parietal and chief cells is described in **Application Sheet C48**

1. Background

ECL cells have been purified mainly from rat or mastomys gastric mucosa, although occasionally ileal mucosa is used. Following dispersal of the mucosal cells by enzymic digestion (usually with Pronase) the first stage in the purification procedure is the isolation of a small cell fraction by centrifugal elutriation. Subsequently the ECL cells are purified by density gradient centrifugation either on a two-layer discontinuous gradient or a simple density barrier. Both Nycodenz® and iodixanol gradients have been used for the gradient step but the density and ionic composition of the solutions are very similar. The current availability of Nycodenz® only as a powder and of iodixanol as a sterile isoosmotic 60% (w/v) solution (OptiPrep™) makes the preparation of the gradient solutions much easier with the latter. Consequently it is the use of OptiPrep™ that is described in the following protocol. Section 5 summarizes some of the Nycodenz® technology. The following protocol is adapted from refs 1-3.

2. Solutions required

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

B. OptiPrep™ diluent: 60 mg/ml BSA, 7.2 mM $MgCl_2$, 90 mM HEPES-NaOH pH 7.4.

C. OptiPrep™ Working Solution (WS) of 50% (w/v) iodixanol: mix 5 vol. of OptiPrep™ with 1 vol. of Solution B.

D. WS diluent: 10 mg/ml BSA, 140 mM NaCl, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, 0.5 mM DTT, 1 mM glucose, 15 mM HEPES-NaOH, pH 7.4

Prepare 100 ml of each of the following stock solutions and keep at 4°C:

1 M HEPES (free acid)	23.8 g
100 mM $MgCl_2 \bullet 6H_2O$	2.03 g
100 mM $MgSO_4 \bullet 7H_2O$	2.46 g
100 mM DTT	1.54 g
1 M NaCl	5.84 g
100 mM $CaCl_2 \bullet 2H_2O$	1.47 g
100 mM glucose	1.8 g*

* Keep glucose at -20°C

Solution B: Dissolve 6 g BSA in 50 ml water; add the following stock solutions: 7.2 ml of $MgCl_2$, 9 ml of HEPES, adjust to pH 7.4 with NaOH and make up to 100 ml.

Solution D: Dissolve 1 g BSA in 50 ml water; add the following stock solutions: 14 ml of NaCl, 1.2 ml of $MgSO_4$, 1 ml of $CaCl_2$, 0.5 ml of DTT, 1 ml of glucose and 1.5 ml of HEPES, adjust to pH 7.4 with NaOH and make up to 100 ml.

3. Protocol

1. Disperse the gastric mucosal cells by Pronase digestion and partially purify the ECL cells by centrifugal elutriation (see ref. 1 for more details).

2. Dilute Solution C with solution D to produce a solution of 10.8% (w/v) iodixanol (approx equivalent to ρ = 1.061 g/ml). For the alternative two-layer gradient make up in addition a 15% iodixanol solution.

3. Layer 1 ml of the ECL cell containing suspension ($2x10^6$ cells) over 10 ml of the 10.8% iodixanol solution in a 15 ml centrifuge tube, or use 5 ml each of the 10.8% and 15% iodixanol (see Note 1).

4. Centrifuge at 1000 rpm for 5 min (at speed), using slow acceleration (400 $rpm.min^{-1}$) and no brake during deceleration. The ECL cells band just above the 10.8% iodixanol layer.

4. Notes

1. As this method isolates the least dense cell from a mixed population containing denser cells, it may be worthwhile considering an alternative flotation strategy. The cells should be suspended in a 15% (w/v) iodixanol solution and layered beneath the 10.8% iodixanol solution. This flotation

strategy has been used very successfully for the isolation of other low-density cells from tissue digests. As an example **see "Dendritic cells" Application Sheet C21 in index**.

5. Nycodenz® technology

Nycodenz® gradients [4-8] contain essentially the same salts, buffer, BSA and DTT composition as the iodixanol solutions. To make up an approximately isoosmotic stock solution of density approx. 50 ml of water in a 150 ml beaker on a heated magnetic stirrer set at approx. 50°C and add 27.6 g of Nycodenz® in small amounts until dissolved. Allow the solution to cool to room temperature; add 16.6 ml of Solution B and then make up to 100 ml with water. Filter sterilize if required. As with iodixanol solutions, make further dilutions with Solution D.

The approach with Nycodenz® has been rather different: most methods use a two-layer gradient in which the ECL band at the interface between these two solutions rather than at the interface below the sample. With solutions of approx. 13.8% and 10.4% (w/v) Nycodenz® [9,10] the ECL cells banded at the interface between the two Nycodenz® solutions. The concentration of the lighter solution is often reduced further: approx 9.2% [4-8] or 7% (w/v) Nycodenz® [11]. There is no obvious reason why this approach could not be extrapolated to the use of OptiPrep™. The density of the 10.4% Nycodenz® solution, through which the ECL cells sediment, is approx 1.058 g/ml, only slightly less dense than the 10.8% iodixanol solution, on which the cells float (see above). It may be necessary to investigate fine changes to the density of any iodixanol solution that may be used when substituting this medium for Nycodenz® in order to optimize the purification.

6. References

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