

OptiPrep™ Application Sheet C48

Fractionation of parietal and chief cells from the 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 gastric ECL cells is described in [Application Sheet C47](#)

1. Background

The acid-secreting parietal cell from rabbit gastric mucosa provides a very useful model for studying both the regulation of ion-transport and intracellular signaling pathways. Since 1986 the most widely used strategy is to purify parietal cells from Pronase/ collagenase-disaggregated gastric mucosa on either continuous or discontinuous Nycodenz® gradients. The purity of the parietal cells is variously reported as 80-95%; sometimes additional purity is achieved by centrifugal elutriation of the Nycodenz®-purified fraction; sometimes the elutriation is executed before the Nycodenz® gradient.

1a. Continuous Nycodenz® gradients

One of the first descriptions of the method was by Chew and Brown [1]; NycoPrep™ 1.15 (an isoosmotic solution of 27.6% Nycodenz®, $\rho = 1.15$ g/ml) was used to prepare the gradient solutions. The NycoPrep™ 1.15 was supplemented with bovine serum albumin (BSA), DTT, KCl, MgSO₄ and buffer, which reduced the density of the solution to 1.139 g/ml. Further dilutions of this Nycodenz® Working Solution were made with an isoosmotic diluent containing NaCl, BSA, DTT, MgSO₄ and buffer, to produce solutions of 1.095, 1.073 and 1.049 g/ml. Gradients were produced from equal volumes (2 ml) of each of the gradient solutions, which were allowed to diffuse to form a continuous gradient. The gastric mucosal cell suspension (2 ml) was layered on top and centrifuged at 1000 g for 8 min [2]. The distribution of material on a continuous gradient is shown in Figure 1. The gradient also resolves the chief cells, which band towards the bottom of the gradient. If chief cells are not required Chew [2] observed that the densest layer may be omitted, permitting the cells to be applied in double the volume; this reduction in cell concentration reduced clumping. Note that NycoPrep 1.15 is no longer commercially available.

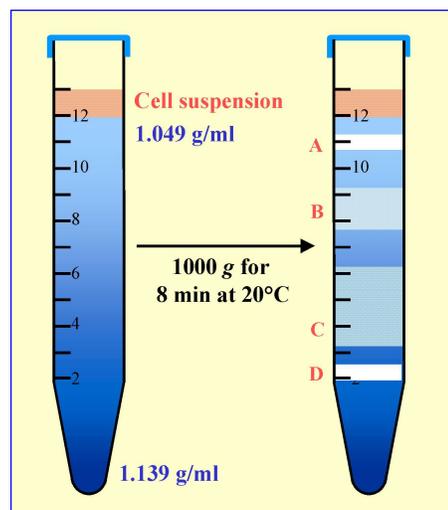


Figure 1: Fractionation of gastric mucosal cells in a continuous Nycodenz gradient [1]. Band A contains 80-95% parietal cells; band B is a diffuse band of less pure parietal cells; band C is mixed cell/mucus; band D contains chief cells.

Benn et al [3] used continuous gradients covering exactly the same density range but added Nycodenz® to the cell suspension to raise its density to just below that of the top of the gradient and used 600 g rather than 1000 g. Cell recoveries of >95% were obtained. The authors also noted that use of Percoll® gave much inferior recoveries and purity of parietal cells and also stimulated acid and cAMP secretion by gastric cells. If elutriation is carried out prior to a continuous gradient, the latter is often 1.04-1.08 g/ml [4-6]

1b. Discontinuous Nycodenz® gradients

Chew [2] used the same solutions of 1.139, 1.095, 1.073 and 1.049 g/ml as a discontinuous gradient and achieved similar results to the continuous gradient. In a discontinuous gradient the parietal cells band at the 1.049/1.073 g/ml interface. Berglindeh [7] also used a four-step gradient of 1.10, 1.075, 1.05 and 1.0375 g/ml, the latter containing the cell suspension. There are several options in which just

two layers are used as the gradient: 8 ml of 1.050 and 5 ml of 1.075 g/ml, overlaid with 2 ml of the cell suspension is a common format [8-10]. Like Berglinth [7], Malinowska [11] suspended the cells in the low-density solution (1.0375 g/ml), layered over a 1.078 g/ml Nycodenz® solution and a 40% (w/w) sucrose solution. In all these two-layer Nycodenz® gradients the parietal cells band at the interface between the Nycodenz® solutions.

1c. Centrifugation conditions

There is a quite diverse range of reported centrifugation conditions: 1000 g for 8 min [1,2], 800 g for 10 min [7], 600 g for 8 min [3] and 200 g for 10 min [8,9]. The higher g-forces, associated with gradients with a maximum density ≥ 1.10 g/ml, may be required for the banding of chief cells; it should be pointed out however that studies with other secreting cells suggest that lower g-forces promote better retention of viability and function.

1d. OptiPrep™ gradients

Chew et al [12-14] have replaced Nycodenz® with iodixanol as the density gradient medium but otherwise kept the continuous gradient density range and other aspects of the centrifugation the same. The authors also reported that iodixanol gradients gave improved purities of parietal cells.

2. Solution selection and gradient preparation

2a. Cell suspension solution

The cell suspension medium is usually a regular balanced salt solution, e.g. 132.4 mM NaCl, 5.4 mM KCl, 5 mM Na₂HPO₄, 1 mM NaH₂PO₄, 1.2 mM MgSO₄ and 1 mM CaCl₂, commonly supplemented with various additions that are added immediately before use: 2 mg/ml BSA, 10 mM glucose, 0.5 or 1.0 mM DTT, 1 mM pyruvate (11 g per 100 ml), 10 mM HEPES, 0.01 mg/ml phenol red and buffered to pH 7.4 with NaOH. Concentrated stocks of these reagents, except the pyruvate and BSA can be kept for several weeks in the refrigerator (see Box 1).

Box 1.

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 KCl	0.74 g
100 mM MgSO ₄ •7H ₂ O	2.46 g
1 M Tris (base)	12.1 g
100 mM DTT	1.54 g
1 M NaCl	5.84 g
100 mM CaCl ₂ •2H ₂ O	1.47 g
100 mM Na ₂ HPO ₄	1.20 g
100 mM NaH ₂ PO ₄	1.42 g

2b. Nycodenz® solutions and gradients

Many of the reported density solutions were prepared from Nycoprep™ 1.15 (an isoosmotic solution of 27.6% Nycodenz® containing 3mM KCl, 0.3 mM CaNa₂-EDTA, 5 mM Tris-HCl, pH 7.4, $\rho = 1.15$ g/ml). This is no longer commercially available so all solutions must be made from Nycodenz® powder. The CaNa₂-EDTA was present only to increase solution stability during autoclaving and may be omitted and the buffer and inorganic salt content may be changed to suit the operator's requirements. Berglinth [7] prepared a solution containing 5.4 mM KCl, 1.2 mM MgSO₄, 15 mM Tris-HEPES, pH 7.4 and 10 mg/ml BSA.

To make 100 ml of this $\rho = 1.15$ g/ml stock solution place 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 0.5 g of BSA followed by the appropriate solutions from Box 1: 5.4 ml of KCl, 1.2 ml of MgSO₄ and 1.5 ml of HEPES, adjust to pH 7.4 with the Tris solution and finally make up to 100 ml with water. Filter sterilize if required.

Further dilutions of the $\rho = 1.15$ g/ml Nycodenz® solution are then made by dilution with a simple isoosmotic diluent such as 132 mM NaCl, 5.4 mM MgSO₄, 0.5 mM DTT, 10 mg/ml BSA, 15 mM HEPES-Tris, pH 7.4 [2], or 128 mM NaCl, 5 mM KCl, 4 mg/ml BSA, 0.3 mM Ca/Na-EDTA, 5 mM Tris-HCl, pH 7.4 [8] or sometimes the cell suspension medium is used [7,11]. Select a gradient from Sections 1a and 1b and prepare the appropriate solutions from the 1.15 g/ml Nycodenz® stock solution and an isoosmotic diluent using the data in Table 1.

Density	Stock Soln. (vol.)	Diluent (vol.)
1.0375	6.4	23.6
1.040	7.0	23.0
1.049	9.0	21.0
1.050	9.1	20.9
1.070	13.3	16.7
1.073	13.8	16.2
1.075	14.2	15.8
1.080	14.9	15.1
1.095	18.5	11.5
1.100	19.5	10.5
1.139	27.8	2.2

Table 1: Density of solutions produced by mixing Nycodenz solution ($\rho = 1.15$ g/ml) with an isoosmotic diluent

Discontinuous gradients: The four-layer format of 1.139, 1.095, 1.073 and 1.049 g/ml [1,2] has been executed with 2 ml of each of the solutions in a 15 ml tube, while the 1.10, 1.075, 1.05 and 1.0375 g/ml gradient described in ref 7 comprised 10ml, 20 ml, 10 ml and 7 ml respectively in a 50 ml tube. In the two-layer format the volume of the low-density solution is usually, but not always, larger than the high-density solution e.g.

8ml and 5 ml in a 50 ml tube [9]. For information on the preparation of discontinuous gradients see [Application Sheet C02](#).

Continuous gradients: In the case of parietal cells these are routinely produced by allowing a discontinuous to diffuse; this is normally accomplished by letting the tube stand vertically in a refrigerator overnight or by capping the tube; smoothly rotating it to a horizontal position and leaving at room temperature for approx. 3 h. The continuous 1.049-1.139 g/ml gradients [1,2] however were generated from layers of 1.139, 1.095, 1.073 and 1.049 g/ml with the tube in a horizontal position overnight at 6-8°C. Continuous gradients may also be produced rather more rapidly using a two-chamber gradient maker or a Gradient Master, both of these methods require the use only of the least dense and the most dense solution. For information on the preparation of continuous gradients see [Application Sheet C02](#).

2c. Iodixanol solutions (Box 2: based on ref 12)

- A. OptiPrep™ (shake gently before use)
- B. Additive solution: 40 mg/ml bovine serum albumin (BSA), 2.0 mM DTT, 9.6 mM KCl, 4.8 mM MgSO₄, 60 mM Tris-HEPES, pH 7.4.
- C. OptiPrep™ diluent: Mix 10 ml of 10x Hank's Buffered Salt Solution (containing Ca and Mg) with 50 ml of Solution B; adjust to pH 7.4 with 100 mM Tris if necessary and make up to 100 ml with water.
- D. OptiPrep™ Working Solution (WS) of 24.4% (w/v) iodixanol ($\rho = 1.134$ g/ml): mix 9 vol. of OptiPrep™ with 11 vol. of Solution C and 2 vol. of water (see Note 1).
- E. WS diluent: 10 mg/ml BSA, 132 mM NaCl, 5.4 mM KCl, 1.2 mM MgSO₄, 0.5 mM DTT, 15 mM Tris-HEPES, pH 7.4

Box 2

Using the stock solutions in Box 1

To make 100 ml of Solution B:

Dissolve 4 g BSA in 50 ml water; add the following stock solutions: 2 ml of DTT, 9.6 ml of KCl, 4.8 ml of MgSO₄, 6 ml of HEPES; adjust to pH 7.4 with 100 mM Tris and make up to 100 ml.

To make 100 ml of Solution E:

Dissolve 1 g BSA in 50 ml water; add the following stock solutions: 13.2 ml of NaCl, 5.4 ml of KCl, 1.2 ml of MgSO₄, 0.5 ml of DTT, and 1.5 ml of HEPES, adjust to pH 7.4 with 100 mM Tris and make up to 100 ml.

Iodixanol solutions have been used for the four-step continuous gradient (or discontinuous) gradient format developed by Chew et al [12-14]. Table 2 describes the preparation of the 1.048, 1.070 and 1.091 g/ml solutions from the 24.4% (w/v) iodixanol working solution (1.134 g/ml) for the preparation of the continuous gradient described in ref 12. It also gives the recipes for other density solutions that might be used as a Nycodenz® substitute. See above for the preparation of both discontinuous and continuous gradients. A discontinuous gradient was also used by Nishi et al [15]; the density of the solutions (1,049, 1,073, 1,095 and 1.139 g/ml) was similar but not identical to that used by Chew et al [12-14]. Separation of the parietal and chief cells using the gradient described has also been used by Noreldin et al [16].

Density	Stock Soln. (vol)	Diluent (vol)
1.040	1.0	2.80
1.048	1.0	2.00
1.050	1.0	1.98
1.070	1.0	1.02
1.073	1.0	0.90
1.075	1.0	0.88
1.080	1.0	0.73
1.091	1.0	0.52
1.095	1.0	0.74
1.100	1.0	0.36

Table 2: Density of solutions produced by mixing OptiPrep™ Solution D ($\rho = 1.134$ g/ml) with an isoosmotic diluent (Solution E)

- ◆ Zhang et al [17] have also used the discontinuous iodixanol gradient strategy
- ◆ Use of a continuous iodixanol gradient was also reported by Singh et al [18] and by Sashidara et al [19].

3. Processing the cell suspension

Pellet the cells from the enzyme digest at 200 g for 8 min and aspirate the supernatant. Resuspend the cell pellet gently in the cell suspension medium (see Section 2a) or in the lowest density gradient solution (see Section 1b) at 0.1-0.15 ml of packed cells per ml. In 12-15 ml tube layer 2 ml of the cells on top of the gradient. In 50 ml tubes use a proportionately larger volume. Reducing the cell concentration will minimize any cell clumping that may occur.

- ◆ For details on preparation of gastric mucosa and its enzymic digestion see refs 1, 2, 12, 20 and 21.

4. Centrifugation

Centrifuge the four-layers gradients at 600-1000 g for 8 min or the two-layer gradients at 200 g for 10 min. See Section 1c for comments about choice of conditions. Use a slow acceleration program if available and allow the rotor to decelerate without the brake. Sudden changes in rpm create vortices in the gradients and the consequent mixing can seriously disturb the gradient. Temperatures vary from approx 10°C to room temperature. Harvest the parietal cells that band at the top of a continuous gradient (see Figure 1) or at the interface below the least dense layer of a discontinuous gradient. Dilute the harvest with at least 2 volumes of Solution E (Section 2c) or the cell suspension medium and harvest by centrifugation at 200 g for 10 min and resuspend the pellet as required.

5. Technical Note

Parietal cells are the least dense of a mixed population of mainly denser cells. In this respect they are not unlike monocytes and dendritic cells. Flotation methods for isolating the least dense cell type have been more successful than sedimentation methods in purifying such cells. Flotation of parietal cells may also provide a purer isolate than sedimentation and avoid subsequent elutriation.

6. References

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