OptiPrep™ Application Sheet C28

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 C31**

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®, ρ = 1.15 g/ml) was used to prepare the gradient solutions. The NycoPrep™ 1.15 was supplemented with bovine serum albumin (BSA), DTT, KCl, $MgSO_4$ 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, $MgSO_4$ 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.

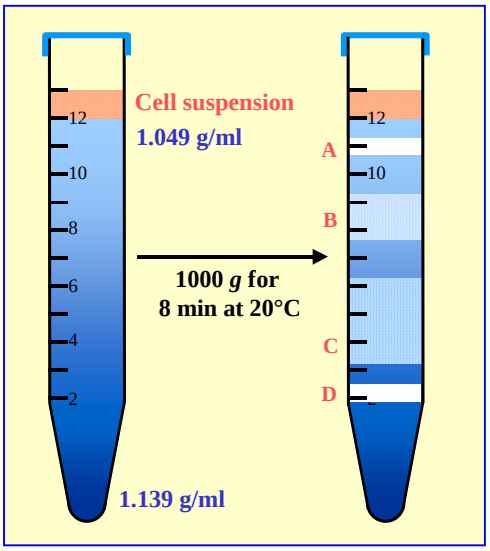

Figure 1: Fractionation of gastric mucosal cells in a continuous Nycodenz gradient [1]. Band A contains 80-95% parietal cells; band B is a diffuse band of less pure parietal cells; band C is a mixed cell/mucus band and band D contains chief cells.

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. Berglindh [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 Berglindh [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 Na_2HPO_4, 1 mM NaH_2PO_4, 1.2 mM $MgSO_4$ and 1 mM $CaCl_2$, 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 $MgSO_4 \bullet 7H_2O$	2.46 g
1 M Tris (base)	12.1 g
100 mM DTT	1.54 g
1 M NaCl	5.84 g
100 mM $CaCl_2 \bullet 2H_2O$	1.47 g
100 mM Na_2HPO_4	1.20 g
100 mM NaH_2PO_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 $CaNa_2$-EDTA, 5 mM Tris-HCl, pH 7.4, ρ = 1.15 g/ml). This is no longer commercially available so all solutions must be made from Nycodenz® powder. The $CaNa_2$-EDTA was present only to increase solution stability during autoclaving and may be omitted and the buffer and inorganic salt content may changed to suit the operator's requirements. Berglindh [7] prepared a solution containing 5.4 mM KCl, 1.2 mM $MgSO_4$, 15 mM Tris-HEPES, pH 7.4 and 10 mg/ml BSA.

To make 100 ml of this ρ = 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 $MgSO_4$ 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 ρ = 1.15 g/ml Nycodenz® solution are then made by dilution with a simple isoosmotic diluent such as 132 mM NaCl, 5.4 mM $MgSO_4$, 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 (ρ = 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 $MgSO_4$, 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 (ρ = 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 $MgSO_4$, 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 $MgSO_4$, 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 $MgSO_4$, 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 (ρ = 1.134 g/ml) with an isoosmotic diluent (Solution E)

- Use of a continuous iodixanol gradient was reported by Singh et al [17] and by Sashidara et al [18].

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, 19 and 20.

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

1. Chew, C. S. and Brown, M.R. (1986) *Release of intracellular Ca^{2+} and elevation of inositol triphosphate by secretagogues in parietal and chief cells isolated from rabbit gastric mucosa* Biochim. Biophys. Acta, **888,** 116-125
2. Chew, C. S. (1990) *cAMP technologies, functional correlates in gastric parietal cells* Meth. Enzymol.**, 191**, 640-661
3. Benn, S.E., Canfield, S.P. and Curwain, B.P. (1987) *Preparation of purified parietal cells from rat using Nycodenz density gradients* J. Physiol., **391**, 9P
4. Roche, S., Gusdinar, T., Bali, J-P-. and Magous, R. (1991) *Biphasic kinetics of inositol 1,4,5-trisphosphate accumulation in gastrin-stimulated parietal cells* FEBS Lett., **282**, 147-151
5. Gros, L., Hollande, F., Thorens, B., Kervran, A. and Bataille, D. (1995) *Comparative effects of GLP-1-(7-36) amide, oxytomodulin and glucagons on rabbit gastric parietal cell function* Eur. J. Pharmacol., **288**, 319-327
6. Nagano, M., Chastre, E., Choquet, A., Bara, J., Gespach, C. and Kelly, P.A. et al (1995) *Expression of prolactin and growth hormone receptor genes and their isoforms in the gastrointestinal tract* Am. J. Physiol. Gastrointest. Liver Physiol., **268**, G431-442
7. Berglindh, T. (1990) *Gastric glands and cells: Preparation and in vitro methods* Meth. Enzymol., **192**, 93-107
8. Lewis, J.L., Goldenring, J.R., Asher, V.A. and Modlin, I.M. (1989) *Pancreastatin: a novel peptide inhibitor of parietal cell signal transduction* Biochem. Biophys. Res. Comm., **163**, 667-673
9. Lewis, J.J., Goldenring, J.R., Asher, V.A. and Modlin, U.M. (1990) *Effects of epidermal growth factor on signal transduction in rabbit parietal cells* Am. J. Physiol. Gastrointest. Liver Physiol., **258**, G476-G483
10. Tsunoda, Y., Modlin, I.M. and Goldenring, J.R. (1993) *Tyrosine kinase activities in the modulation of stimulated parietal cell acid secretion* Am. J. Physiol. Gastrointest. Liver Physiol., **264**, G351-G356
11. Malinowska, D.H. (1990) *Cl^- channel blockers inhibit acid secretion in rabbit parietal cells* Am. J. Physiol. Gastrointest. Liver Physiol., **259**, G536-G543
12. Chew, C. S., Parente, J. A., Chen, X., Chaponnier, C. and Cameron R. S. (2000) *The LIM and SH3 domain-containing protein, lasp-1, may link the cAMP signaling pathway with dynamic membrane restructuring activities in ion transporting epithelia* J. Cell Sci., **113**, 2035-2045
13. Chew, C.S., Okamoto, C.T., Chen, X. and Qin, H.Y. (2005) *IQGAPs are differentially expressed and regulated in polarized gastric epithelial cells* Am. J. Physiol. Gastrointest. Liver Physiol., **288**, G376-G387
14. Chew, C.S., Okamoto, C.T., Chen, X. and Thomas, R. (2005) *Debrin E2 is differentially expressed and phosphorylated in parietal cells in the gastric mucosa* Am. J. Physiol. Gastrointest. Liver Physiol., **289**, G320-G331
15. Nishi, M., Aoyama, F., Kisa, F., Zhu, H., Sun, M., Lin, P., Ohta, H., Van, B., Yamamoto, S., Kakizawa, S., Sakai, H., Ma, J., Sawaguchi, A. and Takeshima, H. (2012) *TRIM50 protein regulates vesicular trafficking for acid secretion in gastric parietal cells* J. Biol. Chem., **287**, 33523–33532

16. Noreldin, A.E., Sogabe, M., Yamano, Y., Uehara, M., Mahdy, M.A.A., Elnasharty, M.A., Sayed-Ahmed, A., Warita, K. and Hosaka, Y.Z. (2016) *Spatial distribution of osteoblast activating peptide in the rat stomach* Acta Histochem., **118,** 109–117
17. Singh, N., Singh, P., Shrivastva, S., Mishra, S.K., Lakshmi, V., Sharma, R. and Palit, G. (2012) *Gastroprotective effect of anti-cancer compound rohitukine: possible role of gastrin antagonism and H^+ K^+-ATPase inhibition* Naunyn-Schmiedeberg's Arch Pharmacol (2012) 385:277–286
18. Sashidhara, K.V., Avula, S.R., Mishra, V., Palnati, G.R., Singh, L.R., Singh, N., Chhonker, Y.S. et al (2015) *Identification of quinoline-chalcone hybrids as potential antiulcer agents* Eur. J. Medicin. Chem., **89**, 638-653
19. Lindström, E., Lerner, U. H. and Håkanson, R. (2001) *Isolated rat stomach ECL cells generate prostaglandin E_2 in response to interleukin-1β, tumor necrosis factor-α and bradykinin* Eur. J. Pharmacol., **416**, 255-263
20. Le Goascogne, C., Sananes, N., Eychenne, B., Gouezou, M., Baulieu. E-M. and Robel, P. (1995) *Androgen biosynthesis in the stomach: expression of cytochrome P450 17α-hydroxylase/17,20-lyase messenger ribonucleic acid and protein, and metabolism of pregnenolone and progesterone by parietal cells of the rat gastric mucosa* Endocrinology, **136**, 1744-1752

7. Acknowledgements

Axis-Shield PoC AS thanks Professor Catherine Chew, Cellular Biology and Anatomy, Medical College of Georgia, Augusta, GA 30912 for her assistance in the preparation of this Application Sheet.

Application Sheet C28; 7th edition, June 2016

AXIS-SHIELD
Innovation for Life