C15 Purification of Islets of Langerhans from porcine pancreas

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml.
- Although this protocol was developed for the isolation of islets from juvenile and adult porcine pancreas [1-3, 34] has been adapted to other species (see Table 2 at end of Application Sheet).
- The OptiPrep™ Applications CD (available from Axis-Shield PoC, AS and its distributors) contains an Index File and associated lists of abstracts. In the Index file of the OptiPrep™ References Folder "find" (Cntrl F) "Pancreatic islets"; the OptiPrep™ Reference Numbers identify abstracts of journal articles, which can be found in File 4 (Cells).

Background

This protocol is based upon an islet isolation method using the University of Wisconsin solution (UWS) as the medium for both collagenase digestion of the tissue at 37°C and for all post-digestion operations (mechanical dispersion, filtration etc) carried out at 0-4°C [1-3]. Some workers may prefer to restrict the use of UWS to the "cold" steps (it may be slightly cytotoxic at 37°C, or it may inhibit digestion in other species); in which case the digestion should be carried out in Hanks Balanced Salt Solutions (HBSS) or in a tissue culture medium such as RPMI (see Note 1). If such a medium is also used for the preparation of the density gradient solutions, modifications will need to be made to the volumes of OptiPrep™ and medium because these culture media have a lower density than that of UWS (see Notes 2 and 3).

The protocol uses a Working Solution containing 30% (w/v) iodixanol (osmolality approx 500 mOsm) produced by mixing OptiPrep™ with an equal volume of double strength UWS (2x). The crude islet suspension is adjusted to ρ = 1.10 g/ml (osmolality approx 380 mOsm) by mixing with the Working Solution and gradient solutions are subsequently prepared by diluting the Working Solution with standard (1x) UWS (see Note 2). The protocol is described as a flow diagram in Figure 1.

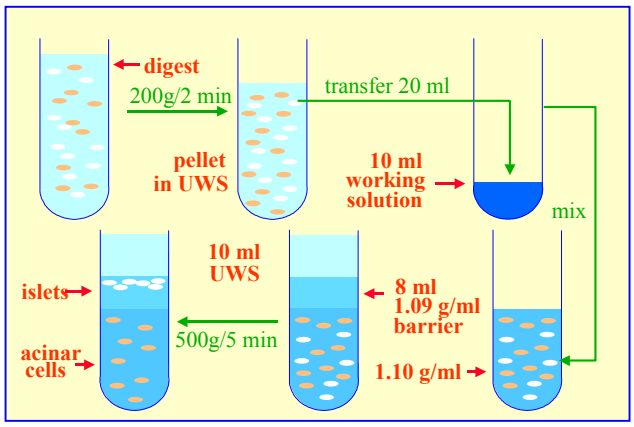

Figure 1 Islet purification flow diagram

- Optimal recoveries may vary with the species, tissue handling procedures and cell suspension medium and may therefore require minor adjustments to the density of the gradient solutions.

Solutions required

A. OptiPrep™ (shake gently before use)

B. OptiPrep™ diluent: UWS(x2).

C. Diluent for gradient solutions: UWS (see Notes 6 and 7).

D. Working Solution (WS, ρ = 1.206 g/ml):mix equal volumes of Solutions A and B and transfer 10ml aliquots to 50 ml conical centrifuge tubes. Keep these at 4°C.

E. Low-density barrier solution (ρ = 1.090 g/ml): mix 10 ml WS with 26.36 ml of UWS and keep at 4°C (see Notes 8 and 9).

Solution B: For 2 litres, dissolve 143.3 g of lactobionic acid (200 mM) in 1250 ml of distilled water, then adjust to pH 7.0 with 5 M KOH before adding the following in the order given (see Note 4):

- 13.6 g KH_2PO_4 (50 mM)
- 2.4 g $MgSO_4$ (10 mM)
- 71.3 g raffinose (60 mM)
- 0.27 g allopurinol (1 mM) (see Note 5)
- 3.68 g glutathione [reduced] (6 mM)
- 5.34 g adenosine (10 mM)
- 200 g pentastarch (100 g/l) (see Note 6)

Adjust with 5 M NaOH to pH 7.4 and make up to 2 litres.

Solution C: Dilute Solution B 1:1 with water

Filter sterilize Solutions B and C and store at 4°C.

Protocol

1. Digest the pancreatic tissue with collagenase in UWS (or other chosen medium) at 37°C, then carry out all subsequent operations (mechanical dispersion, filtering etc) in UWS at 0-4°C.

2. Centrifuge the digest for 2 min at 200g at 4°C and gently resuspend the pellet in UWS and make up to volume (a multiple of 20 ml) with this medium (e.g. 10-12 ml of packed tissue pellet in 80 ml).

3. Transfer 20 ml of digest suspension into each of the prepared centrifuge tubes containing 10 ml of WS and mix rapidly but gently by repeated inversion or pouring repeatedly between two centrifuge tubes.

4. Layer 8 ml of the low-density barrier solution over the suspension and top up the tube with 10 ml of (1x) UWS.

5. Centrifuge at 500g for 5 min at 4°C (see Note 10). Islets band at the top interface; acinar tissue remains in the load zone (see Figure 1 and Note 11).

6. Harvest the islets using a syringe and wide-bore metal cannula; dilute with an equal volume of (1x) UWS and pellet at 200g for 4 min.

Notes

1 If a medium such as HBSS or RPMI is used for the cold isolation steps, the tissue should be pre-incubated in cold UWS for 60 min before addition of the Working Solution. The gradient however may require significant adjustment of density and perhaps osmolality [2]

2 UWS(x2) has a density of 1.092 g/ml. Double strength HBSS or RPMI have a lower density (approx 1.012 g/ml), consequently the amount of single-strength medium required to produce solutions of the appropriate density will require modifying (see Notes 3 and 8).

3 See OptiPrep™ Application Sheet C1 for more information about preparing density gradient solutions for mammalian cells.

4 Neutralization of the lactobionic acid should be carried out slowly and carefully.

5 Allopurinol is kept at the same concentration as in UWS (1x) as higher concentrations are difficult to dissolve.

6 Contact Mr B Henriksen (Axis-Shield PoC), fax, 47 22 04 20 01; e-mail, bjh@no.axis-shield.com regarding commercial sources of pentastarch powder and UWS (1x).

7 UWS may be purchased commercially or it can be prepared using half the concentration of the reagents in Solution B (except allopurinol which should be at the same concentration). Alternatively it may be prepared by diluting Solution B with an equal volume of water (check pH is still 7.4), but note that the allopurinol concentration will be half that normally in UWS (1x).

8 It may be necessary to modulate the density of this layer [2] according to the isolation method that is used or if islets are purified from other species. Table 1 gives the volumes of UWS and Working Solution required to produce solutions of different densities.

UWS (ml)	Density (g/ml)
22.65	1.095
31.03	1.085
37.06	1.080
45.17	1.075

Table 1 Density of solutions prepared from mixing 10 ml of Working Solution (ρ = 1.206 g/ml) and different volumes of UWS

9 It may be an advantage to produce the barrier solution in RPMI, this can act as a preliminary means of washing the islets free from UWS, as they float to the upper interface. Good results have been obtained with barrier

solutions prepared by diluting OptiPrep™ with RPMI or RPMI containing 10% serum: 3.2 ml of OptiPrep™ and 8.8 ml of RPMI gives a solution of ρ = 1.090 g/ml; if RPMI containing 10% serum is used the density is approx 1.092 g/ml.

10 Recently it has been suggested that the recovery, purity, resistance to fragmentation and insulin response to glucose are all improved by reducing the RCF to 100g [18]. Longer centrifugation times may consequently be required.

11 Unacceptable levels of acinar tissue contamination in the islet layer normally imply that the density of the barrier layer is too high and should be reduced.

- The methodology is now widely used for porcine islets; it has also been adapted and extended to other species and modified for use in the Cobe 2991 centrifuge rather than tubes. Table 2 summarizes these applications chronologically.

Table 2 Bibliography of published papers reporting the use of iodixnol gradients for islet purification

Animal source	Centrifugation	Research topic	Ref #
Pig	Tube	Islet recovery, purity and viability	1
Pig	Tube	Islet preservation	2
Pig	Tube	Islet integrity and culture	3
Human	Tube	Islet integrity and recovery	4
Pig	Tube	Islet viability for transplants	5
Human	Tube	Islet viability and purity	6
Pig	Cobe	Large scale preparation /allograft function	7
Pig	Cobe	Modification to xenogeneic reaction to pig islet cells	8
Pig	Tube	Islet culture and transplantation in nude mice	9
Pig	Cobe	Reversal of diabetes in primates after transplantation	10
Pig	Cobe	Role of MAP kinases following isolation of islets	11
Pig	Cobe	*In vitro* xenorecognition of islet cells by lymphocytes	12
Pig	Tube	Suppression of early rejection of islets in monkeys	13
Rat	Tube	Covalent attachment of PEG	14
Pig	Cobe	Loss of insulin release caused by xenogeneic mononuclear cells	15
Pig	Cobe	Yield, purity and *in vitro* function	16
Rat	Tube	Secretion of preptin derived from pro-insulin-like growth factor	17
Pig	Cobe	Use of low speed (100g) improves yields and quality	18
Pig	Cobe	Decreased insulin release by co-incubation with spleen cells	19
Human	Cobe	Improved function after prolonged *in vitro* culture	20
Pig	Cobe	*In vitro* recognition and impairment of function by baboon immune cells	21
Human/rat	Tube	Vesicular inhibitory amino acid transporter distribution	22
Human	Cobe	Dynamic perifusion to assess metabolic and functional viability	23
Rat	Tube	Continuous measurement of oxygen consumption	24
Pig	Cobe	Insulin treatment of murine recipients preserves β cell function	25
Human/non-human primate	Tube	Oxygen-charged static two-layer method for pancreas preservation	26
Pig	Cobe	Isolation from young market pigs; islet morphology in porcine donor	27
Pig	Cobe	Microchimerism and transmission of endogenous retrovirus	28
Pig	Cobe	Modulation of cellular and humoral xenogeneic reactions	29
Human	Cobe	Cationic lipid and polymer-based gene delivery	30
Human/non-human primate	Tube/Cobe	Improved yields after two-layer preservation and endogenous trypsin inhibition	31
Pig	Tube	T cell-specific immunosuppression effect on survival in monkey	32
Human	Cobe	Maximization of yield, culturing and immunosuppression	33
Pig	Tube	Islet purification protocol	34
Rat	Tube	Improved yield and function with ductal injection of UWS	35
Rat	Tube	Regulation of ATP/ADP	36
Human	Cobe	Cultured islet transplantation from two-layer preserved pancreases	37
Pig	Cobe	Transmission of encephalomyocarditis virus	38

- A number of reviews of islet methodology for diabetes treatment have also been published (39-44).

References

1. Van der Burg, M. P. M., Basir, I. & Bouwman, E. (1998) *Transplant. Proc*., **30**, 362-363.
2. Van der Burg, M. P. M., Basir, I., Zwaan, R.P. & Bouwman, E. (1998) *Transplant. Proc*., **30**, 360-361
3. Van der Burg, M. P. M., Zwaan, R. P. & Bouwman, E. (1998) *Horm. Metab. Res*., **30** A23.
4. Van der Burg, M. P. M., Ranuncoli, A., Molano, R., Ringers, J., Bouwman, E. & Ricordi, C. (1998) *Acta Diabetologia*, **35**, 247.
5. Rijkelijkhuizen, J. K. R. A., Bouwman, E. & Van der Burg, M. P. M. (1999) *Cell Transplant.*, **8**, 173.
6. Van der Burg, M. P. M., Ranuncoli, A., Molano, R., Kirlew, T., Ringers, J., Bouwman, E. Terpstra, O. T. & Ricordi, C. (1999) *Cell Transplant.,* **8**, 184.
7. Matsumoto, S., Zhang, H. J., Gilmore, T., van der Burg, M.P.M., Sutherland, D. E. R. & Hering, B. J. (1998) *Transplant.*, **66**, S30.
8. You, S., Gouin, E. & Sai, P. (1998) *Diabetologia*, **41**, 955-962.
9. Van der Burg, M. P. M., Rijkelijkhuizen, J. K. R. A., Zwaan, R. P. & Bouwman, E. (1999) *Cell Transplant.*, **8**, 18.
10. Matsumoto, S., Shibata, S., Kirchhof, N., Hiraoka, K., Sageshima, J., Zhang, X. W.., Gilmore, T., Ansite, J., Zhang, H. J., Sutherland, D. E. R. & Hering, B. J. (1999) *Transplant.*, **67**, S220.
11. Paraskevas, S., Aikin, R., Maysinger, D., Lakey, J. R. T., Cavanagh, T. J., Hering, B., Wang, R. & Rosenberg, L. (1999) *FEBS Lett.*, **455**, 203-208.
12. Lalain, S., Chaillous, E., Gouin, E. & Sai, P. (1999) *Diabetologia*, **42**, 330-335.
13. Rijkelijkhuizen, J. K. R. A., Bouwman, E., Van der Burg, M. P. M., Ringers, M. P., Ossevoort, M. A., Kuhn, E. M., Frost, P. & Jonker, M. (2000) *Cell Transplant.*, **9**, 909-912.
14. Panza, J. L., Wagner, W. R., Rilo, H. L. R., Rao, R. H., Beckman, E. J. & Russell, A. J. (2000) *Biomaterials*, **21**, 1155-1164.
15. Lalain, S., Clemenceau, B., Gouin, E. & Saç, P. (2001) *Human Imunol.*, **62**, 607-614.
16. Krickhahn, M., Meyer, T., Buchler, C., Thiede, A. & Ulrichs, K. (2001) *Ann. Transplant.*, **6**, 48-54.
17. Buchanan, C. M., Phillips, A. R. J. & Cooper, G. J. S. (2001) *Biochem. J.*, **360**, 431-439.
18. Shibata, S., Sageshima, J., Hiraoka, K., Zhang, H., Koyama, K., Sutherland, D. E. R. & Hering, B .J. (2001) Abstracts of the Int. Pancreas Islets Transplant. Assoc., Innsbruck, p5.
19. You, S., Rivereau, A-S., Gouin, E. & Sai, P. (2001) *Clin. Exp. Immunol.*, **125**, 25-31.
20. Gaber, A. O., Fraga, D. W., Callicutt, C. S., Gerling, I. C., Sabek, O. M. & Kotb, M. Y. (2001) *Transplant.*, **72**, 1730-1736.
21. Lalain, S., Gianello, P., Gouin, E. & Sai, P. (2001) *Transplant.*, **72**, 1541-1548.
22. Chessler, S. D., Simonson, W. T., Sweet, I. R. & Hammerle, L. P. (2002) *Diabetes*, **51**, 1763-1771.
23. Sweet, I. R., Cook, D. L., Wiseman, R. W., Greenbaum, C.A., Lernmark, A., Matsumoto, S., Teague, J. C. & Krohn, K. A. (2002) *Diabetes Tech. Therapeut.*, **4**, 67-76.
24. Sweet, I. R., Khalil, G., Wallen, A. R., Steedman, M., Schenkman, K. A., Reems, J. A., Kahn, S. E. & Callis, J. B. (2001) *Diabetes Tech. Therapeut.*, **4**, 661-672.
25. Pakhomov, O., Honiger, J., Gouin, E., Cariolet, R., Reach, G. & Darquy, S. (2002) *Cell Transplant.*, **11**, 721-728.
26. Matsumoto, S., Rigley, T. H., Qualley, S. A., Kuroda, Y., Reems, J. A. & Stevens, R. B. (2002) *Cell Transplant.* **11**, 769-777.
27. Krickhahn, M., Buhler, C., Mayer, T., Thiede, A. & Ulrichs, K. (2002) *Cell Transplant.*, **11**, 827-838.
28. Clemenceau, B., Jegou, D., Martignat, L. & Sai, P. (2002) *Diabetologia*, **45**, 914-923.
29. You, S., Gouin, E. & Sai, P. (2002) *Clin. Exp. Immunol.*, **127**, 412-422.
30. Mahato, R. I., Henry, J., Narang, A. S., Sabek, O., Fraga, D., Kotb, M. & Gaber, A. O. (2003) *Mol. Therapy*, **7**, 89-100.
31. Matsumoto, S., Rigley, T. H., Reems, J. A., Kuroda, Y. & Stevens, R. B. (2003) *Am. J. Transplant.*, **3**, 53-63.
32. Rijkelijkhuizen, J. K. R. A., Haanstra, K. G., Wubben, J., Tons, A., Roos, A., van Gijlswijk-Janssen, D., Ringers, J., Bouwman, E. & Jonker, M. (2003) *Transplant.*, **76**, 1359-1368.
33. Hering, B. J., Kandaswamy, R., Ansite, J. D., Eckman, P. M., Nakano, M., Sawada, T., Matsumoto, I., Ihm, S-H, Zhang, H-J., Hunter, D. W. & Sutherland, D. E. R. (2003) *IPITA 2003,* abstract 012.
34. Van der Burg, M. P. M. & Graham, J. M. (2003) *The Sci. World J.*, **3**, 1154-1159.
35. Sawada, T., Matsumoto, I., Nakano, M., Kirchhof, N., Sutherland, D. E. R. & Hering, B. J. (2003) *Transplant.*, **75**, 1965-1969.
36. Sweet, I. R., Cook, D. L., DeJulio, E., Wallen, A., Khalail, G., Callis, J. & Reems, J. A. (2004) *Diabetes*, **53**, 401-409.
37. Hering, B. J., Kandaswamy, R., Harmon, J. V., Ansite, J. D., Clemmings, S. M., Sakai, T., Paraskevas, S., Eckman, P. M., Sageshima, J., Nakano, M., Sawada, T., Matsumoto, I., Zhang, H. J., Sutherland, D. E. R. & Bluestone, J. A. (2004) *Am. J. Transplant.*, **4**, 390-401.
38. Brewer, L., LaRue, R., Hering, B. J., Brown, C. & Njenga, M. K. (2004) *Xenotranplant.*, **11**, 160-170.
39. White, S. A., James, R. F. L., Swift, S. M., Kimber, R. M. & Nicholson, M. L. (2001) *Diabetes*, **18**, 78-103.
40. Stevens, R. B. (2001) *Clin. Diabetes*, **19**, 51-60.
41. Shapiro, A. M. J., Nanji, S. & Lakey, J. R. T. (2003) *Immunol. Rev.*, **196**, 219-236.
42. Kandeel, F., Smith, C. V., Todorov, I. & Mullen, Y. (2003) *Pancreas*, **27**, e63-e78.
43. Shapiro, A. M. J. (2003) *J. Am. Soc. Nephrol.*, **14**, 2214-2216.
44. Shapiro, A. M. J. & Ricordi, C. (2004 *Am. J. Transplant.*, B4**, 295-298.**

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