OptiPrep™ Application Sheet C15

Purification of Islets of Langerhans from porcine, primate and rodent pancreas in a discontinuous iodixanol gradient

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- The **OptiPrep™ Mini-Review (MC05)** "Islet purification" provides a protocol review and full bibliography: to access return to the initial list of Folders and select "**Mini-Reviews**"
- 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

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 Solution (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).

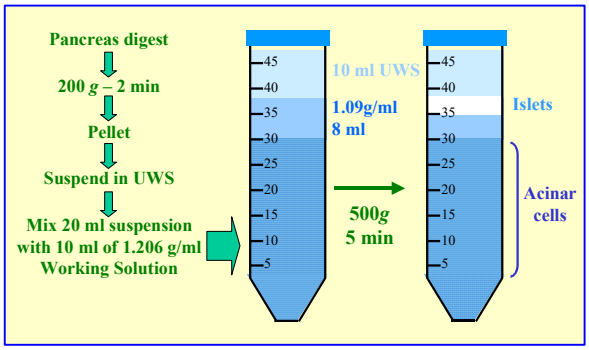

Figure 1: Islet purification flow diagram; for further details see text

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.

- Optimal recoveries may vary with the species and pre-gradient procedures and may require minor adjustments to the gradient. Section 5 contains some information on rat islet isolation.

2. Solutions required

A. OptiPrep™ (shake gently before use)

B. OptiPrep™ diluent: UWS(x2).

C. Diluent for gradient solutions: UWS (see Note 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, and 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.

3. 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 200 *g* 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 500 *g* 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 200 *g* for 4 min.

4. 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. For more information about preparing density gradient solutions for mammalian cells see **Application Sheet C01**.
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. For sources of pentastarch (hydroxyethylstarch) powder contact Fresenius Kabi AG, Germany (www.fresenius-kabi.com) or B. Braun, USA (www.bbraunusa.com).
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 100 *g* [4]. 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.

5. Rat islets

Some rat islet preparation methods incorporate a useful technical operation that is worth noting briefly here. Panza et al [5] first concentrated the cells at an interface by centrifuging the tissue digest over 5 ml of OptiPrep™ (250 *g* for 15 min). The supernatant was removed (except for 2-3 ml) and the residual material in the tube was mixed, then iodixanol solutions of 1.135, 1.120, 1.096 g/ml and saline layered on top. After centrifugation at 800 *g* for 30 min the islets banded at the 1.096 g/ml – saline interface. This is a convenient way of keep the gradient volume to a relatively small volume without pelleting the cells first. Buchanan et al [6] used a similar strategy but after mixing the residual material a 10-25% v/v OptiPrep™ gradient was layered on top and centrifuged at 800 *g* for 25 min.

6. References

1. Van der Burg, M. P. M., Basir, I. and Bouwman, E. (1998) *No porcine islet loss during density gradient purification in a novel iodixanol in University of Wisconsin solution* Transplant. Proc., **30**, 362-363
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7. Acknowledgements

Alere Technologies AS thanks Dr M.P.M. van der Burg, Department of Surgery, University Hospital, Leiden, NL 2300RC, Netherlands for his help and comments in the preparation of this Application Sheet.

Application Sheet C15; 8th edition, January 2018

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