C15 Purification of Islets of Langerhans from porcine, primate and rodent pancreas

- **OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml**
- For links to other relevant files click on the double blue arrow in the following text
- See Section 5 for full bibliography

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).

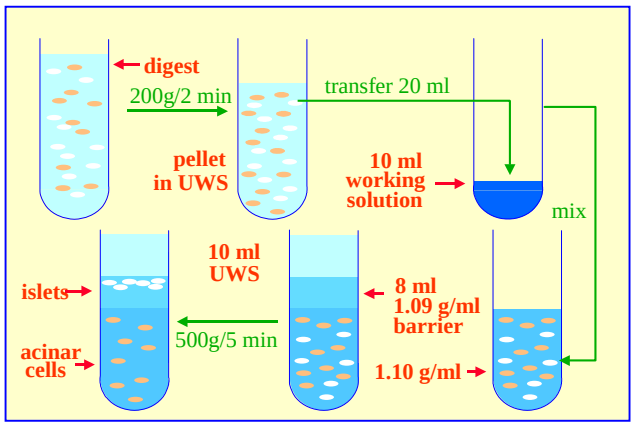

Figure 1: Islet purification flow diagram; for further details see text

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, tissue handling procedures and cell suspension medium and may therefore require minor adjustments to the density of the gradient solutions.

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 OptiPrep™ Application Sheet C1. ➔➔
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 100g [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. Bibliography

- Table 2 summarizes the OptiPrep™ bibliography on human and non-human primate islets
- Table 3 summarizes the OptiPrep™ bibliography on porcine islets
- Table 4 summarizes the OptiPrep™ bibliography on rodent islets

Table 2: Papers reporting the use of iodixanol gradients for human and non-human primate islet purification

Source	Research area	Research topic	Ref. #
Human	Gene Delivery	Cationic lipid and polymer-based gene delivery (C)	5
		Gene delivery of endothelial growth factor (C)	6,7
	In vitro culture	Improved function after prolonged *in vitro* culture (C)	8
		Preservation of *in vivo* function after culture in serum-free media (C)	9
	Transplantation	Transplantation from two-layer preserved pancreases (C)	10,11
		Transplantation (single donor) into Type 1 diabetes patients (C)	12
		Transplantation from non-heart-beating donors (C)	13-17
	Transport studies	Vesicular inhibitory amino acid transporter distribution (T)	18
	Yield, viability and function	Donor age, effect on function related to ATP generation	19
		Islet integrity and recovery (T)	20
		Islet viability and purity (T)	21
		Improved yields after two-layer preservation and trypsin inhibition (T,C)	22
		Improved yields from obese donors, for transplantation (C)	23
		Oxygen-charged static two-layer method for pancreas preservation (T)	24
		Perifusion (dynamic) to assess metabolic and functional viability (C)	25
		Reagent endotoxin levels in islet purification (C)	26
Non-human primate		Improved yields after two-layer preservation and trypsin inhibition (C, T)	22
		Oxygen-charged static two-layer method for pancreas preservation (T)	24

(C): Method adapted to the Cobe 2991 centrifuge, (T): Centrifugation in tubes

Table 3: Papers reporting the use of iodixanol gradients for porcine islet purification

Research area	Research topic	Ref. #
Immune reactions to transplantation	*In vitro* recognition/impairment of function by baboon immune cells (C)	27
	In vitro xenorecognition of islet cells by lymphocytes (C)	28
	Suppression of early rejection of islets in monkeys (T)	29
	T cell-specific immunosuppression effect on survival in monkey (T)	30
	Xenogeneic reaction to pig islet cells (C)	31
	Xenogeneic reactions, modulation of cellular and humoral (C)	32
Insulin release	Insulin release loss caused by xenogeneic mononuclear cells (C)	33
	Insulin release decrease by co-incubation with spleen cells (C)	34
Transplantation in diabetes treatment	Diabetes, reversal of, in primates after transplantation (C)	35,36
	Insulin treatment of murine recipients preserves β cell function (C)	37
	Microcapsular transplantation (C)	38
Viruses	Microchimerism and transmission of endogenous retrovirus (C)	39,40
	Virus infection, human coxsackie B-5 virus	41
	Virus transmission, encephalomyocarditis virus	42
Yield, viability and function	Centrifugation at low speed (100g) improves yields and quality (C)	4
	Cobe 2991 closed system for rapid islet isolation (C)	43
	Islet culture and transplantation in nude mice (T)	44,45
	Islet preservation, integrity and culture (T)	2,3
	Islet preservation and purification (C)	17
	Islet recovery, purity, function and viability (T)	1,46
	Islet viability for transplants (T)	47
	Large scale preparation /allograft function (C)	48
	MAP kinases, role of, following isolation of islets (C)	49
	Yield, purity and *in vitro* function (C) 4.431	50,51
	Young market pigs, isolation from; islet morphology (C)	52

(C): Method adapted to the Cobe 2991 centrifuge, (T): Centrifugation in tubes

Table 4 Papers reporting the use of iodixanol gradients for rodent islet purification

Animal type	Research topic	Ref. #
Mouse	Islet neogenesis associated protein transgenic mice in β cell pathology	53
	ER molecular chaperone gene $P58^{IPK}$ mutation and β cell failure	54
Rat	γ-Aminobutyric acid transporter association with glucose-regulated expression	55
	ATP/ADP, regulation of	56
	β Cell imaging agents	57
	Calcium influx mediation of glucose-stimulated oxygen consumption	58
	Islet quality assessment by cytochrome c reduction and oxygen consumption	59
	Oxygen consumption, continuous measurement of	60
	PEG, covalent attachment	61
	Preptin derived from pro-insulin-like growth factor, secretion of	62
	Purification in iodixanol, improvement compared to Ficoll	63
	Two-layer preservation method improvement in islet yield	64
	Vesicular inhibitory amino acid transporter distribution	65
	Yield (improved) and function with ductal injection of UWS	66

- Reviews of islet methodology for diabetes treatment have also been published - see refs 67-73.

6. References

To access abstracts of refs 1-26 (File CA15a) click on the double blue arrow. ➔➔
To access abstracts of refs 27-52 (File CA15b) click on the double blue arrow. ➔➔
To access abstracts of refs 53-66 (File CA15c) click on the double blue arrow. ➔➔

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