UW Islet Tx Research Program
Version: 1/24/05

Animal Islet Isolation Protocol

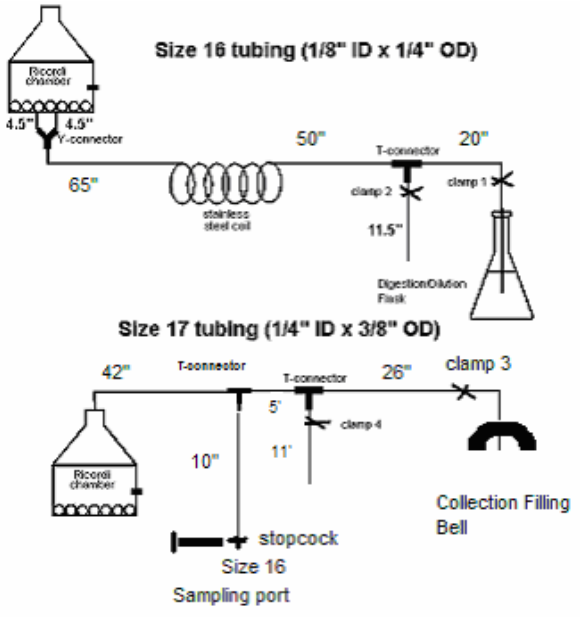
Materials Inventory and Preparation Instructions

Surgical Instrument and Digestion Apparatus Pack

Item	Quantity	Check
Nalgene PP bin	1	
Medium SS tray	1	
Small SS tray w/lid	1	
Hammer SS	1	
Metzenbaum scissors blunt	1	
Metzenbaum scissors sharp	1	
Gemini clamp	1	
Mayo scissors	1	
Classic Crile forceps	2	
Delicate Crile forceps straight	3	
Delicate Crile forceps curved	4	
Dressing forceps	2	
Children's dressing forceps	1	
Ricordi chamber (250 ml)	1	
SS screen 381 um	1	
SS screen 450 um	1	
Silicon nitride ½" balls	7	
Heating coil	1	
100 ml wide mouth graduate	1	
1 L Erlenmyer flasks	2	
Surgical gauze in a steilization pouch	1	
Digestion circuit L/S 16	1	
Digestion circuit L/S 17	1	
Thermometer clamps	4	
Three prong fixed clamps	3	
Three prong adjustable clamp	1	

Preparation of Surgical Instruments and Digestion Apparatus

1) Wipe down the counter with IPA.
2) Place two layers of surgical wrapping on the counter.
3) Place the autoclave tray (or PP bin) on the wrapping on the counter.
4) Place the stainless steel pans inside the autoclave tray (or PP bin).
5) Place the surgical instruments inside the instrument tray, cover, and place in the autoclave tray.
6) Place the Ricordi digestion chamber with screen and 5 marbles, stand, and heating coil in the autoclave tray.
7) Place 20 pieces of 4”x4” gauze in an autoclave pouch, seal, and place in the autoclave tray.
8) Aluminum foil wrap the top of a 100 ml wide-mouth graduated cylinders and place in the autoclave tray.
9) Aluminum foil wrap the top of one 1 L Erlenmeyer flask and one 400 ml Pyrex beaker and place in the autoclave tray.
10) Cut the L/S 16 tubing into the following sizes (2 x 4.5 in, 1 x 11.5 in, 1 x 10 in, 1 x 20 in, 1 x 50 in, 1 x 65.0 in). Following the diagrams shown below, connect tubing to the Y and T connectors, place pinch clamps, place cable ties on all connections, and place tubing set into a sterilization pouch; seal and label the pouch and place in the autoclave tray.
11) Cut the L/S 17 tubing into the following sizes (1 x 5 in, 1 x 11.0 in, 1 x 26 in, 1 x 42.0 in). Following the diagrams shown below, connect tubing to the 3-way stopcock and T-connector, place cable ties on all connections and place into a sterilization pouch; seal and label the pouch and place in the autoclave tray.
12) Place three fixed 3-prong ring clamps, 1 adjustable 3-prong clamp, and 4 thermometer clamps in the autoclave tray.
13) Place a cleaned media collection bell and tubing adaptor in an autoclave pouch, seal, and place in the autoclave tray.
14) Wrap the autoclave tray as for a sterile surgical instrument pack.
15) Tape the outside of the package with autoclave indicator tape and label with contents, date, and name.
16) Sterilize the surgical instrument pack by autoclave using a PreVac cycle at 121^{0}C for 20-30 minutes.
17) Store the complete/sealed Bin in room G24A.



Disposables Pack

Item	Quantity	Check
Disposable scalpel	1	
Monotherm temp probe	4	
Monotherm temp cables	2	
2.0 Suture	1	
3 Way stopcock	1	
5 cc syringe	2	
10 cc syringe	2	
60 cc syringe	4	
18 ga Angiocath	2	
20 ga Angiocath	2	
24 ga Angiocath	2	
18 ga Needle	5	
20 ga Needle	5	
Petri dishes 10 x 35	1 sleeve	
Petri dishes 10 x 35 w/grid	1 sleeve	
Petri dishes 100 mm	1 sleeve	
Tissue culture plate, 12 well	1 pack	

Islet Purification Pack

Item	Quantity	Check
Classic Crile forceps straight (on Cobe)	5	
Blood processing bags	2	
COBE couplers	2	
60 cc syringe	2	
Fenwal Transfer bag 600 ml	2	

Reagents

Item	Quantity	Check
2 % FBS in HBSS (Flushing solution)	1 L	
2.5% FBS in UW (Islet suspension/wash soln.)	1 L	
2.5% FBS in RPMI (Dilution solution)	5 L	
CMRL 1066 w/10% FBS (Islet culture)	1 L	
1x HBSS	1 L	
Decontamination soln 1		
Decontamination soln 2		
Decontamination soln 3		
Biocoll BL (130 ml)	1	
Biocoll CL 1 (130 ml)	1	
Biocoll CL 2 (140 ml)	1	
Krebs buffer (static incubation)	1 L	
FDA stock	1	
PI stock	1	
DPBS	1 L	
Dithizone soln	50 ml	
1x PBS sheath buffer	25 L	
Euro-Ficoll 1.037	75 ml	
Euro-Ficoll 1.096	75 ml	
Euro-Ficoll 1.108	75 ml	

Complete directions for media preparation can be found in UW03 ISL-SOP-002.

Islet Isolation Procedure

1. Adult Beagle or Rhesus – surgically euthanized with pancreas perfused *in situ* with cold UW solution.
2. Pancreas is stored in 250 ml of ice-cold UW solution and transported directly to the islet isolation lab.
3. Islets are isolated using a modified version of the Miami Diabetes Research Institute protocol for canine islet isolation.
    a. Pancreas cannulated and then distended and digested with Liberase CI in 1x HBSS (Canine [0.25 g], Rhesus [0.125 g] /500 ml total vol.) in a Ricrodi digestion chamber and recirculation circuit at 37^{o}C for up to 30 minutes.
    b. Monitor digest progression by taking samples, staining with dithizone solution and observing with the inverted microscope.
    c. Dilution of pancreas digest is accomplished with 6-7 L of 10% RPMI and collection is directly into 500 ml conical centrifuge tubes containing 30 ml of 2.5% FBS in RPMI.
    d. Digest is pelleted by centrifugation at 1000 rpm for 2 minutes at 4^{0}C.
    e. After the spin, the supernatants are poured into a waste beaker or aspirated into the waste flask. The pellets are gently resuspended and combined into a separate 500 ml conical tube, which is stored on ice until all of the collected digest has been centrifuged.
    f. The final collected digest is washed in 2.5% FBS in UW solution and pelleted by centrifugation at 1000 rpm for 2 minutes at 4^{o}C.
    g. The supernatant is discarded and the pellet is resuspended in 2.5% FBS in UW solution according to the packed tissue volume.
        i. 15 – 20 ml of tissue – bring to 100 ml with 2.5% FBS in UW and run gradient on the COBE.
        ii. 30 – 50 ml of tissue – bring to 200 ml with 2.5% in UW and run two gradients on the COBE.
    h. Refer to UW03 ISL-CP for gradient loading and COBE centrifugation instructions.
    i. After 2-3 minutes of centrifugation at 2000 RPM collect 17, 30 ml fractions into 50 ml conical centrifuge tubes (DO NOT STOP THE COBE).
    j. Take 100 µl samples of each fraction, transfer to a 10 x 35 mm counting dish containing a small quantity of PBS, stain with dithizone, and observe with the inverted microscope for islets.
    k. Determine islet purity, and yield in IEQ manually. Data are recorded on the Data Summary Sheet.

Modified Islet Purification Method – Discontinuous Gradient Centrifugation

1. Pancreas digest is washed in Eurocollins solution.
2. Digest is resuspended in 1.108 g/ml Euro-Ficoll gradient at a ratio of 1:11 (tissue: Ficoll) and 12 mL is aliquoted per 50 ml conical centrifuge tubes.
3. Ten mL of 1.096 g/ml and ten ml of 1.037 g/ml Euro-Ficoll are then added to each discontinuous gradient.
4. The gradients are centrifuged at 900 xg for 12 – 15 minutes and the islets collected at the interfaces.
5. Purified islets are washed in CMRL culture media, pooled, and counted.

10 mL 1.037 g/mL EF
10 mL 1.096 g/mL EF
12 mL Islets in 1.108 g/ml EF