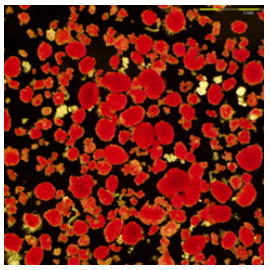
Jan 25, 2019

IsletCore Equipment Setup for Human Islet Isolation



Endocrinology

In 2 collections

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Human Cell Atlas Metho... CIRTNR2FIC 1 more workspace

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OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

Created: August 08, 2018

Last Modified: January 25, 2019

Protocol Integer ID: 14588

Guidelines

For all solutions refer to protocol: **Human Islet Isolation Media Preparation** protocol
Perform all proceedures aseptically.

Materials

MATERIALS

- Silicon Nitride Marbles **Biorep Diabetes Catalog #**SN-01
- Ricordi Chamber **Biorep Diabetes Catalog #**RC-600-MDUR/WM-533
- Temperature Probe **Biorep Diabetes Catalog #**TC-02
- Wire mesh - 533 µm **Biorep Diabetes Catalog #**600-WM-533
- Ricordi Chamber Ring **Biorep Diabetes Catalog #**RC-ORING-600
- RICORDI® ISLET ISOLATOR **Biorep Diabetes Catalog #**RI5-115
- RICORDI ISOLATOR TUBING SET **Biorep Diabetes Catalog #**RI5-TUBSET-WB
- DISSECTION COOLING TRAY **Biorep Diabetes Catalog #**DCT-01
- CONICAL COOLING TRAY **Biorep Diabetes Catalog #**CCT-01
- Perfusion Apparatus **Biorep Diabetes Catalog #**PER5-115
- PERFUSION TUBING SET - without heating coil **Biorep Diabetes Catalog #**PER05-TUB-01
- PERFUSION TRAY **Biorep Diabetes Catalog #**PERTRAY-SS-01
- Yankauer - Suction Handle Flexible Regular Capacity Clear **Cardinal Health Catalog #**PK61

Room setup

1
- Turn on both biosafety cabinets (BSC) 20 minutes prior to use. Be sure to disinfect the BSC work surfaces with 70% ethanol.
- Turn on COBE 2991 chiller to 4°C.
- Turn on chilling recirculating bath set to 4ºC (at least 4 hours prior to isolation start)
- Turn on centrifuge and set to program 1 (282xg, 4°C, 2 min). Be sure the centrifuge is equipped with centrifuge buckets designed to acoomadate 250 mL centrifuge tubes.
- Turn on Biorep perfusion system.
- Place biomedical waste receptacles in lab (1 box and 1 pail).
- Fill ice tray and bucket with ice and place in refrigerator

Documentation

2 Fill out relevant information related to the isolation using the donor information sheet.

HOPE donor information sheet.docx

Prepare the Isolation worksheet.

Isolation worksheet.xlsx

Setup Auto-isolator

3 Turn on the auto-isolator by turning on the power swtich at the back of the unit. Once logged in Press the "Isolator Protocol Wizard".
Allow the shaker arm to initiate
Click on the Next icon to continue to the “load isolator protocol” page

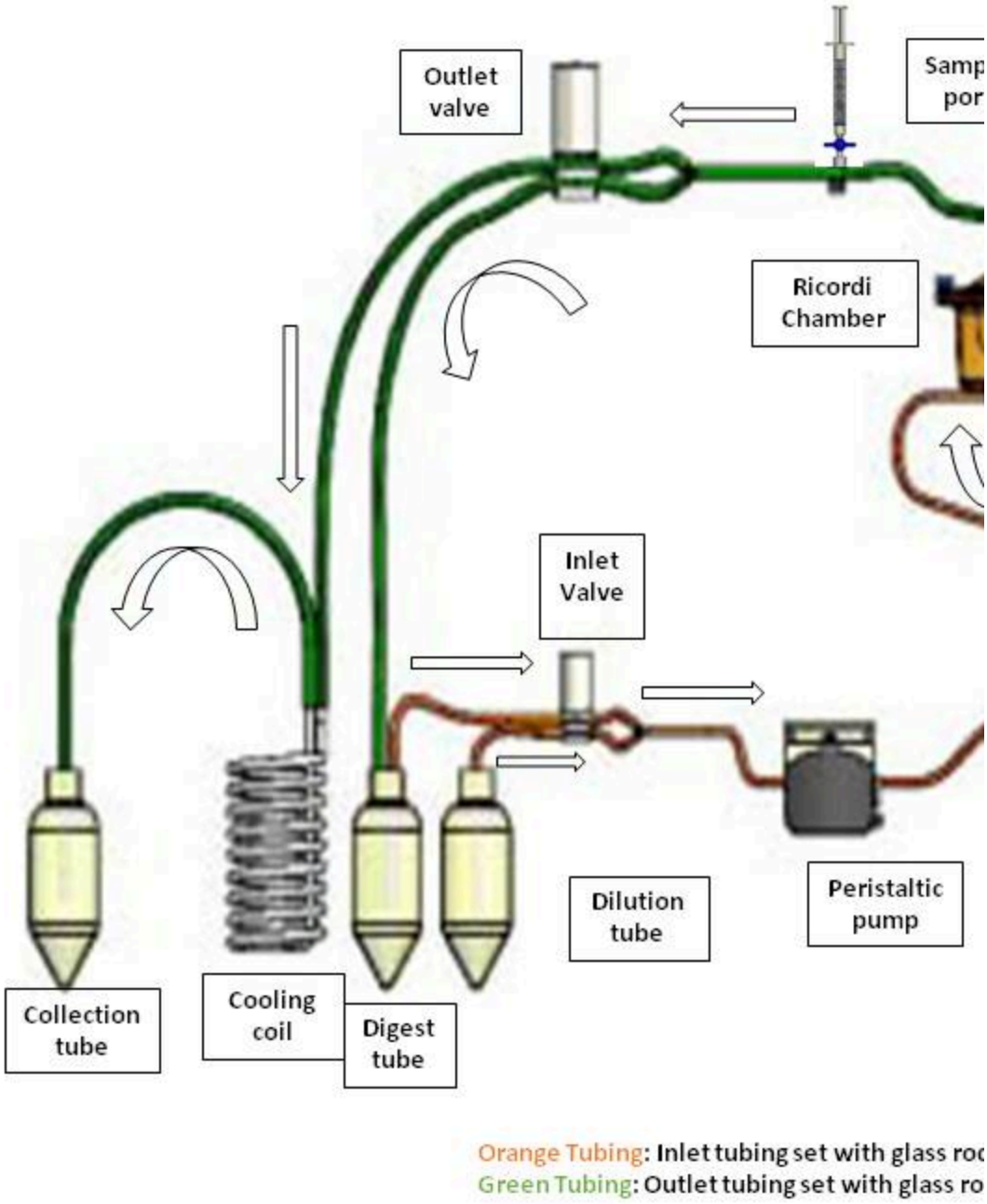
4 Select the “standard” protocol that has will load the pre-programmed steps for the protocol. This protocol is user defined.

		Stage name	Flow rate (ml/min)	Chamber Temp (oC)	Cooler (oC)	Inlet Valve (On/Off)	Outlet Valve (On/Off)	Motion Profile (Vf,Vs,Rf,Rs)
	1	Prime	200	37	4	Off	Off	
	2	Pause	0	0	4	Off	Off	
	3	Fill	300	37	4	Off	Off	

	4	Digest 1	300	37	4	Off	Off	(140,110,140,80)
	5	Digest 2	150	37	4	Off	Off	(140,110,140,80)
	6	Collection 1	300	0	4	On	On	(140,110,140,80)
	7	Pause	0	0	4	On	On	
	8	Collection 2	300	0	4	On	On	(140,110,140,80)
	9	Stop	0	0	4	Off	Off	

- **Vf-Vertical frequency**
- **Vs-Vertical stroke**
- **Rf-Rotational frequency**
- **Rs-Rotational stroke**

5
- Setup the digest/collection tubing lines as illustrated in an applicable BSC utilizing aseptic technique throughout procedure:
- Unwrap sterilized Ricordi chamber, secure top chamber to bottom chamber with steel clamp
- Unwrap sterilized stainless steel heating coil and assemble to inlet tubing set and glass rods.
- Unwrap sterilized stainless steel cooling coil and assemble to outlet tubing set and glass rods
- Connect sterile thermocouple to Ricordi chamber temperature port. Plug thermocouple into thermocouple monitor.
- Connect a 5cc syringe to the sampling port on the outlet tubing set.
- Place glass digestion rods in priming solution until the first step in the isolation profile
- Install the now assembled tubing sets/Ricordi Chamber to the inlet valve, peristaltic pump, heating interface, infrared temperature controller, pressure transducer, shaking arm, outlet valve, and the cooling bath filled with 70% Ethanol.



- Initiate the isolator by using HBSS/priming solution in step 1 on the protocol “priming”.
- Observe the system for any problems (leaks, temperature, etc).
- Allow the system circulate until just before the completion of the pancreas perfusion in the Islet Isolation protocol.

IMG_6621.jpg

Set up the surgical area in preparation for cannulation and perfusion.

6

- Setup the surgical/perfusion equipment in an applicable BSC utilizing aseptic technique throughout procedure:
- Unwrap outer wrapping of sterilized cutdown tray and fold the wrapping underneath the tray as a sterile barrier.
- Connect cold water “in” and “out” lines to ports on cutdown tray. Tighten with wrench. Unclamp circulating cold water lines and turn on cold water bath (set to operate at 3°C).
- Remove air inside cutdown tray by raising water outlet port to allow air to escape.
- Open surgical pack (kidney bowl, mosquito forceps (x6), tissue forceps - toothed and non toothed, drummond forceps, silicon nitride marble set (9 marbles), micro scissors, Metzenbaum scissors (x2), 8×11 stainless steel tray) and open all sterile instruments and disposables for use during the decontamination and perfusion.
- Open cutdown tray screens
- Place decontamination pack (3 sterile glass 500ml beakers) and pancreas container inside BSC.
- Open wrap of decontamination pack and pour decontamination medias 1, 2, and 3 into three separate sterile beakers.
- Label a sterile 15 mL conical tube as “Biopsy” with Isolation number (R#).
- Remove wrap from sterilized perfusion tray inside BSC.
- Connect cold water “in” line to bottom port on left side of perfusion chamber, and “out” line to top port.
- Connect the line in and line out water lines to perfusion tray inlet and outlet ports.
- Open perfusion tubing set and attach it to the perfusion tray and perfusion apparatus.

IMG_6619.jpg

- Block temperature probe port on perfusion tray with 3cc syringe.
- Pour 500 mL of HBSS solution onto cutdown tray.

IMG_6620.jpg

Perfusion appartus protocol

7 The perfusion appartus should be pre-programed using the following profile:

	Step	Time	Pressure SP	Pressure SP	Temp SP
			mm Hg	mm Hg	Tray
		min	Channel A	Channel B	

	1	240	50	50	4
	2	240	100	100	4
	3	240	175	175	4

Set up tissue collection following the digest

8

- Place circulating conical tube chilling block into BSC and connect cold water “in” line to bottom port and “out” line to top port. Tighten with wrench.
- Turn on cold water bath (set to operate at 3°C).
- Remove air inside chilling block by raising water outlet port to allow air to escape.
- Place 12 tubes filled with 100ml of Dilution 1 media into the cold block
- Transfer inlet rod into 2L bottle of Dilution 2 media
- Transfer outlet rod into first tube in cold block in preparation of tissue dilution following the digestion step of the isolation.

IMG_6843.jpg

Set up Yankhauer and tissue collection bottle

9

Obtain bucket with ice and place in BSC
Place sterile 1L glass media bottle with 300ml Wash 1 solution on ice.
Assemble suction apparatus as follows:

- Place suction stand in BSC.
- Connect one of the vacuum lines to the suction canister and the house vaccum source.
- Add a second vaccuum line to the inlet of the cannister and the other end through the opening of the side of the BSC
- Sterily attach a Yankauer suction tip to the inlet vacuum line and clamp it vertically tip down on the stand.
- Open vacuum valve.