TARUMANAGARA HUMAN CELL TECHNOLOGY (THCT) LABORATORY
FACULTY OF MEDICINE, TARUMANAGARA UNIVERSITY
Jl. Letjen S. Parman No. 1; Jakarta 11440 INDONESIA
Phone. +62 21 5696 3254, Fax. +62 21 5696 7325, Email. thctlab11@gmail.com

STANDARD OPERATING PROCEDURE (SOP)	Document Num.: S-001
	Total pages: 3
	Release date: 11 August 2015
Procurement of Rat Pancreas Organ	Effective date: 11 August 2015
	Version: 2
	First draft: IAZ (German)

Materials / Equipment:

1. Electric and blade razor
2. Sterile surgery devices and bulldog clamp
3. Sterile surgery tray and needle/tape
4. Sharp container
5. Carbogen gas bottle
6. Aervent gas filter
7. Sterile silicon tubing for carbogen gas
8. Sterile silicon tubing for perfusion
9. Cartridge pump (Masterflex)

Solutions / Media:

1. Ketamil injection (Ilium, Troy laboratories pty limited Australia)
2. Xylazil (Ilium, Troy laboratories pty limited Australia)
3. Heparin injection
4. **For perfusion with EGTA only**: 130 ml washing buffer with 0.5 mM EGTA (Sigma Cat #E4378) supplemented with 1% of AB/AM (Sigma Cat #A5955) (see Appendix)
5. **For perfusion with EGTA and Enzyme**: 60 ml washing buffer with 0.5 mM EGTA and 1 % AB/AM; 70 ml Enzyme solution, 50 U/mL Collagenase type IV (Gibco #17104-019)in DMEM Low glucose (Biowest Cat #L0064) contain or completed with 2 mM L-Glutamin (Sigma Cat #G7513), 1 mM Na-pyruvat (Sigma Cat #8636), 5-10mM HEPES (Sigma Cat # H0887) and 1% AB/AM (Sigma Cat #A5955) (See Appendix)

Consumables:

1. Sterile nitrile gloves
2. Surgery mask and surgery hat
3. 1 mL syringe with needle
4. Sterile cotton balls
5. Sterile aluminium foil
6. Small and big autoclavable bags
7. Used newspaper
8. Sterile gauge
9. 70 % ethanol spray

Work sheet:

Date : _____
Name of officer : _____
Type/Sex : _____
Start at : _____

sticker

Researcher:

A. BODY WEIGHT AND BLOOD GLUCOSE MEASUREMENT

☐ Weigh the rat with digital balance _____ (target: 150-200 gr)

☐ Measure the blood glucose level of the animal with glucose meter *One Touch*. Blood sample is obtained from the tail vein of the animals for glucose assay. _____ (target: 100-200 mg/dl)

B. ANESTHESI AND SHAVING

☐ Anesthesia the rat with Ketamil (80 mg/kg) ____ (target: 0.15 ml) and Xilazil 2% (5 mg/kg weight body) ____ (target: 0.05 ml) intraperitoneally.

☐ Shave the rat's abdominal hair then place the rat on the surgical pad (*Note: Transfer the rat to the Operation Room*).

C. PANCREAS ORGAN PROCUREMENT

Note:

☐ **Without Perfusion (Skip step 3-11) – technique A**
☐ **Perfusion with EGTA (Skip step 9-10) – technique B**
☐ **Perfusion with EGTA and Enzyme (Do all the steps below) – technique C**

☐ 1. Decontaminate the operation area with providone iodine solution. Cut abdominal skin and inner muscle with a scissor, starting from the bottom, make diagonal cut to the left and right side of the animal (diagonally), up to but not through the diaphragm. Pull the abdominal skin and muscle over the top of the animal, exposing the abdomen.

☐ 2. Using sterile fingers, sweep the intestine and stomach to the right side of the animal, exposing the veins. Cover the bottom and left side of the animal with sterile gauze or cloth.

☐ 3. Inject the heparin solution _____ (target: 0.3 ml), to avoid blood coagulation, into the Inferior Vena Cava (IVC) using 1 ml syringe. Hint: Pull back the IVC while injecting the needle into the vein. Wait for one minute before performing the perfusion.

☐ 4. Construct the sterile perfusion equipment according to the following scheme. Switch on the peristaltic pump to start pumping 0.5 mM EGTA Solution, pre-warmed (37°C) and bubbled with carbogen gas, target volume ____ (technique A: 130 ml; technique B: 60 ml), from the lowest rate _____ (target: 3 ml/min). Start: _____

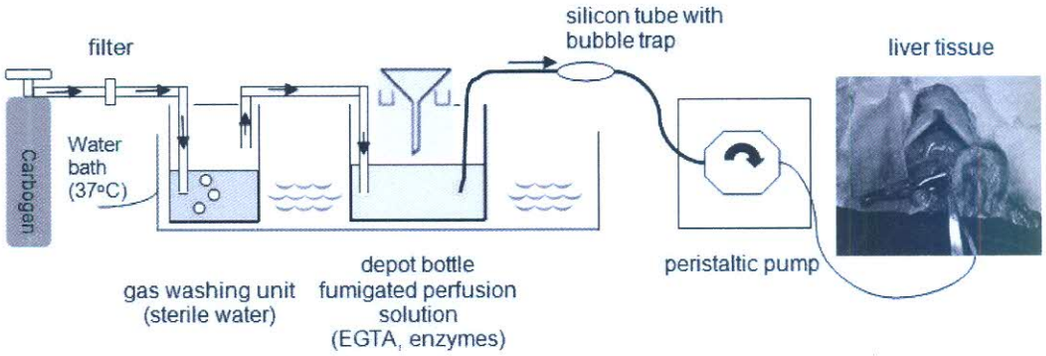


Fig. 1. Schematic drawing of the perfusion equipment.

☐ 5. Insert the iv cat needle (27G) into the portal vein and fix the iv cat needle position with a *Bulldog Clamp*.

☐ 6. Increase the flow rate to _____ (target: 10 ml/min) and then to _____(target: 15 ml/min).

☐ 7. Cut the Inferior Vena Cava (critical point!).

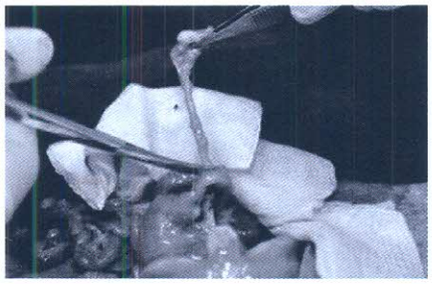
☐ 8. Pancreas should start to look pale and swell. Stop the perfusion after all the EGTA solution ran out _____(target: 5 min).
Rat's Pancreas observation:

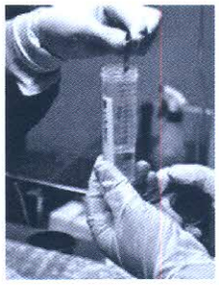
☐ 9. Switch the perfusion tubing to continue the perfusion with freshly prepared _____ (target: 70 ml) pre-warmed _____ (10 U/ml) Collagenase Solution, adjust the flow rate to _____ (target: 10 ml/min).

☐ 10. Pipette warm DMEM into the abdominal area carefully with to flush the blood and to keep the organ warm during perfusion. Block the IVC (1-2 times) to make sure the perfusion went wel, the liver organ will swell.
(Note: Total perfusion time should be less than 10 minutes)

☐ 11. When the perfusion is completed, remove the *bulldog clamp* and iv cat needle.

☐ 12. Resects carefully the pancreas organ as much as possible.





☐ 13. Place the pancreas into a centrifuge tube containing _____ (target: 15 ml) of PBS cold solution. *(Note: Transfer the organ to the processing room)*

☐ 14. After organ procurement, the abdomen wall is closed (using PDS II 5-0 and silk 4-0 running sutures.

TARUMANAGARA HUMAN CELL TECHNOLOGY (THCT) LABORATORY
FACULTY OF MEDICINE, TARUMANAGARA UNIVERSITY
Jl. Letjen S. Parman No. 1; Jakarta 11440 INDONESIA
Phone. +62 21 5696 3254, Fax. +62 21 5696 7325, Email. thctlab11@gmail.com

STANDARD OPERATING PROCEDURE (SOP)	Document Num.: S-002
	Total pages: 8
	Release date: 11 August 2015
Isolation of Rat Islet Cells	Effective date: 11 August 2015
	Version: 2
	First draft: IAZ (German)

Materials/Equipment

1. 2 Empty Petri dishes (sterile / disposable)
2. 10 ml Pipette + Pipette Boy
3. 2 CASO agars
4. Pancreas' forceps set (1 pointy forceps + 1 big stainless spatula)
5. 2 scalpels
6. 2 centrifuge tubes and rack
7. Tea strainer (sterile)
8. 2 Beaker glasses, 250 ml (sterile)
9. Cell sieve 100um (BD Falcon)
10. Sterile microcentrifuge tubes
11. Digital balance
12. Microtubes 1.5 ml (sterile)
13. Improved Neubaeur cell counting chamber with cover
14. Centrifuge
15. 70 % ethanol spray

Solution/Media:

1. 10 ml PBS Solution (Sigma #D8662) +1% Ab/Am (Sigma #A5955)
2. 30 ml Enzyme Solution, 10 U/mL Collagenase Type I (Gibco #17100-017) with 0,01 mg/mL Hyaluronidase (Sigma #H4272) (See Appendix)
3. 30 ml Williams E Media completed with 2 mM L-Glutamin (Sigma Cat #G7513), 1 mM Na-pyruvat (Sigma Cat #S8636), 1% AB/AM, and 10% FBS (GIBCO #10270)
4. Tryphan blue 1:2 (0.36 %)(Sigma cat #T8154)

Seeding

1. Coated Matrices
2. Plate 12 well
3. Matrix set (1 matrix forceps, 1 ring forceps, 1 small stainless spatula)
4. Sterile ring

Transport Back

1. Plates 12 well
2. Matrix set (1 matrix forceps, 1 ring forceps, 1 small stainless spatula)
3. Plastic transport (sterile)
4. 10 ml Pipette + Pipette Boy
5. Transport Box

D. ISLET CELLS OF LANGERHANS ISOLATION

sticker

Date of Surgery : _____
Rat/Sex : _____/ _____
W. of Pancreas : _____
Weight of Rat : _____

Researcher:

1. Sterility test in laminar flow:

☐ Pipette _____ ml (target: 0.3-0.5 ml) of transport solution into a CASO-agar plate, and incubate the agar at 32 °C.

☐ Put the pancreas tissue with sterile forceps into a 2nd CASO-agar plate (weight the organ and roll it over the agar) and incubate the agar at 32 °C.
Macroscopic evaluation:

2. Pancreas cells isolation:

☐ Transfer the pancreas tissue to a sterile petri dish with _____ ml pre-warmed (37° C) PBS (with 1% Antibiotic Antimicotic) (target: 10-15 ml)

☐ Aspirate the PBS from the petri dish with a pipette, replace with _____ ml (target: 10-15 ml) pre-warmed (37° C) enzyme solution (10 U/ml) and cut the tissue with two sterile scalpels as small as possible.

☐ Microscopic observation:

☐ Incubate the desintegrated pancreatic tissue for _____ min (target: 10-15 min) at 37 °C and 5% CO_2.

☐ Microscopic observation:

☐ Sieve the desintegrated pancreatic islet, with the aid of a serological pipette, through a tea strainer (in 250 ml beaker glass) and rinse the strainer thoroughly with _____ ml (target: 15-20 ml) enzyme solution (10 U/ml)

☐ Centrifuge the cell suspension for 5 min at _____ G (target max: 130 G).

☐ Aspirate the supernatant and re-suspend the pellet with _____ ml (target 10-15 ml) Williams E Media, completed with 10% FBS.

☐ Additional: If there is any cells clumping, sieve the clumping cells through a nylon strainer 100 µm (BD Falcon) that placed on a 50 ml centrifuge tube. Wash the strainer with _____ ml (target: 5-10 ml) Williams E Media completed with 10% FBS.

☐ Centrifuge the cell suspension for 5 min at _____ G (target max: 130 G).

☐ Aspirate the supernatant, re-suspend the pellet with _____ ml (target: 1 ml) Williams E Media, completed with 10% FBS.

☐ Determine the cell number and viability by the Trypan blue exclusion method.

☐ Incubate the cells until further processing at 37 °C and 5% CO_2 incubator.

3. Islet cells counting:

Total volume of cells suspension: _____ ml
Dilution factor : _____
Viable cells : _____/_____ squares
Dead cells : _____/_____ squares
Viable + dead cells : _____/_____ squares

Pancreatic cells per ml : _____
Total pancreatic cells : _____
Viability : _____%

Formula
Cells / ml =
(viable + dead) cells/square x dilution factor x common factor (Improved Neubauer: 10^4)
Total pancreatic cells = cells/ml x total volume
Viability = viable cells/(viable + dead) cells

E. ISLET CELLS SEEDING TO THE MATRICES

Name of officer : _____
Rat : _____
Date: _____ Time: _____

1. Cells seeding

☐ Mix the pancreas cell suspensions (mixture volume = _____ ml)

☐ Pipette _____ ml cell suspension (target: 0.4 ml) drop by drop slowly onto _____ a matrix that placed in a 12-wells plate. Incubate cell-containing matrix for _____ (target: 10 minutes).

☐ Add_____ ml (target: 0.6 ml) Williams E Med completed with FBS in the well.

☐ Incubate it at 37 °C and 5% CO_2.

2. Media changing

Date: _____ Time: _____

☐ Aspirate all supernatant from the well; total volume aspirated _____ ml

☐ Count the left over cells in the supernatant

☐ Add _____ ml (target: 1 ml) pre-warmed Williams E Media completed with FBS 10%, the well.
Attention: Pipette the media slowly through the wall of the well. DO NOT pipette the media directly on top of the matrix!

☐ Put the sterile rings on the matrix

☐ Continue the incubation (48 hrs) at 37 °C and 5% CO_2.

3. Media change leftover cells counting:

Total volume of cells suspension: _____ ml
Dilution factor : _____
Viable cells : _____/_____ squares
Dead cells : _____/_____ squares
Viable + dead cells : _____/_____ squares

Pancreatic cells per ml : _____
Total pancreatic cells : _____
Viability : _____%

F. TRANSPORT BACK

Name of officer : _____
Rat : _____
Date of surgery : _____

Date: _____ Time: _____
Total incubation time: _____ h (= time for the adhesion of cells into matrices).

1. Preparing matrices for the transport:

☐ Remove the rings from the well with a sterile forceps.

☐ Transfer the matrix from the wells using a sterile spatula and a sterile forceps carefully into the new 12-wells plate.

☐ Add ____ ml (target: 0.4 ml) pre-warmed Williams E Media (completed with FBS).

☐ Put the plate into a transport bag and seal it with a tape.

☐ Keep the plate in incubator before transportation. By the time of transportation, put the plate in a transport box filled with hot packs, to keep the temperature around 35-37°C during transportation (to Bogor).

☐ The plate will be transport back to the lab. Aspirate the leftover supernatant, performed the sterility test on CASO agar and (leftover) cells counting.

2. Leftover counting (before transport):

Total volume : _____
Dilution factor : _____
Viable cells : _____/_____ squares
Dead cells : _____/_____ squares
Viable + dead cells : _____

Pancreatic cells per ml : _____
Total pancreatic cells : _____
Viability : _____%

3. Leftover counting (after transport):

Total volume : _____
Dilution factor : _____
Viable cells : _____/_____ squares
Dead cells : _____/_____ squares
Viable + dead cells : _____

Pancreatic cells per ml : _____
Total pancreatic cells : _____
Viability : _____%

Adherence Calculation:

cells seeded (pancreas) : _____

cells on media change leftover : _____

cells on leftover (before transport) : _____

cells on leftover (after transport) : _____

_____

Adhered cells : _____

Adherence of cells:

adhered cells /seeded cells X 100% = _____ %

STERILITY TEST RESULT

CASO-Agar	**Bacterial growth after 3 days**	
Pancreas (**Transfer media**)	☐ Yes	☐ No
Pancreas (**Organ**)	☐ Yes	☐ No
Media left over	☐ Yes	☐ No

Completion sterility tests

Date:_____ Signature:_____

APPENDIX: **BUFFER AND SOLUTIONS**

Washing buffer

☐ Weigh required salts in accordance with the target volume;

Salts	Final concentration	Quantity / 1 L vol.	Quantity / ___ L vol
NaCl (Sigma cat# S9625)	137 mM	8 g	_____ g
KCl (Sigma cat# P4504)	2.68 mM	0.2 g	_____ g
$Na_3PO_412H_2O$ (Sigma cat# S7778)	0.7 mM	0.266 g	_____ g
Glucose (Sigma cat# G8270)	10 mM	1.8 g	_____ g

☐ Pour all salts in a beaker glass, add sterile Millipore water up to 90% of target volume: _____ L

0.5 mM Ethylene Glycol Tetraacetic Acid / EGTA Solution (ES)

☐ Add _____mL of 10 mM sterile EGTA solution in _____mL sterile washing buffer

☐ Add _____mL AB/AM for 1% final concentration

50 U/ml COLLAGENASE SOLUTION (CS) (for perfusion)

☐ Weight collagenase type IV _____ mg to get 50 U/mL for _____ mL complete low glucose DMEM media (depends on units content on each batch)

☐ Add collagenase into 50 mL centrifuge tube containing 20 mL DMEM media. Pipet and mix until completely dissolve.

☐ Sterilize the mixture through 0.22 uM syringe filter into 250 mL glass bottle containing _____ mL sterile DMEM media.

☐ Add _____mL AB/AM (final conc 1%) and store at 4°C

☐ Add _____mL of 1M Hepes for 5-10 mM final concentration and store at 4°C

Note: It's better to use freshly prepared or at least one day before of collagenase solution

Enzyme (Collagenase and Hyalorunidase) Buffer Solution

☐ Weigh required salts in accordance with the target volume

Salts	Final concentration	Quantity / 1 L vol.	Quantity / ____ L vol
NaCl (Sigma cat# S9625)	137 mM	8 g	_____ g
KCl (Sigma cat# P4504)	2.68 mM	0.2 g	_____ g
$Na_3PO_412H_2O$ (Sigma cat# S7778)	0.7 mM	0.266 g	_____ g
Glucose (Sigma cat# G8270)	10 mM	1.8 g	_____ g
$CaCl_2$ (Sigma cat# C5670)	5 mM	0.735 g	_____ g

☐ Pour all salts **except** $CaCl_2$ in a beaker glass, add sterile Millipore water up to 90% of target volume: _____ mL. Stir until dissolve.

☐ Pour $CaCl_2$ in separate beaker glass and add 5% of target volume: _____ mL. Stir until dissolve.

☐ Add $CaCl_2$ solution into salts solution. Milky appearance will be formed.

☐ To get clear appearance and all salts dissolved, adjust pH at _____ (target 6.0, **don't less**) using 1 M HCl _____ (target 1550 µl / 1 L).

☐ Add _____ mL of 1M HEPES (Sigma cat# H0887) (target 5-10 mL/1L or final concentration 5-10 mM) to reach pH at _____ (target 7.0 – 7.2)

☐ Add sterile Millipore water up to 100% of target volume: _____L. Stir and mix well.

☐ Check the osmomolarity (target 290 – 310 m.osmol): _____m.osmol

☐ Filter sterilize solution using 0.2 µm steritop filter. Store in refrigerator (4-6°C) until use.

10 U/ml COLLAGENASE SOLUTION (CS) (for isolation)

☐ Weight collagenase type I _____ mg to get 10 U/mL for 30 mL Enzyme solution (depends on unit content on each batch).

☐ Add collagenase into 50 mL centrifuge tube containing 30 mL Enzyme buffer. Pipet and mix until completely dissolve.

☐ Add _____µL of Hyaluronidase (final conc 0.01 mg/ml)

☐ Sterilize the mixture through 0.22 uM syringe filter into new centrifuge tube and store at 4°C

REPUBLIK INDONESIA
KEMENTERIAN HUKUM DAN HAK ASASI MANUSIA

SURAT PENCATATAN CIPTAAN

Dalam rangka pelindungan ciptaan di bidang ilmu pengetahuan, seni dan sastra berdasarkan Undang-Undang Nomor 28 Tahun 2014 tentang Hak Cipta, dengan ini menerangkan:

Nomor dan tanggal permohonan	:	EC00201808885, 12 April 2018
Pencipta		
Nama	:	**Siufui Hendrawan**
Alamat	:	Jl. Letjen S. Parman No.1, Jakarta, Dki Jakarta, 11440
Kewarganegaraan	:	Indonesia
Pemegang Hak Cipta		
Nama	:	**Siufui Hendrawan**
Alamat	:	Jl. Letjen S. Parman No.1, Jakarta, Dki Jakarta, 11440
Kewarganegaraan	:	Indonesia
Jenis Ciptaan	:	**Buku Panduan/Petunjuk**
Judul Ciptaan	:	**Procurement Of Rat Pancreas Organ And Isolation Of Rat Islet Cells**
Tanggal dan tempat diumumkan untuk pertama kali di wilayah Indonesia atau di luar wilayah Indonesia	:	1 Maret 2017, di Jakarta
Jangka waktu pelindungan	:	Berlaku selama hidup Pencipta dan terus berlangsung selama 70 (tujuh puluh) tahun setelah Pencipta meninggal dunia, terhitung mulai tanggal 1 Januari tahun berikutnya.
Nomor pencatatan	:	000105444

adalah benar berdasarkan keterangan yang diberikan oleh Pemohon.
Surat Pencatatan Hak Cipta atau produk Hak terkait ini sesuai dengan Pasal 72 Undang-Undang Nomor 28 Tahun 2014 tentang Hak Cipta.

a.n. MENTERI HUKUM DAN HAK ASASI MANUSIA
DIREKTUR JENDERAL KEKAYAAN INTELEKTUAL

Dr. Freddy Harris, S.H., LL.M., ACCS.
NIP. 196611181994031001