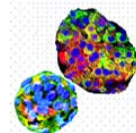


SOP



Title:	Differentiation of PANC1 cells				
Protocol #:	1.3	Submitted:	050510	Approved:	200510
Category:	CC	Author(s): ¹	MRU, MVJ	Checked by:	AAH

Reagents:

1. DMEM; Gibco (Catalog # 11885-084)
2. F-12 medium; Cellgro (Catalog # 10-080-CV)
3. BSA (ICN Biomedicals inc. Catalog # 152401, lot 3506F)
4. ITS (Gibco # 51300-044)
5. Taurine (Sigma catalog # T-8691; FW 125.1)
6. GLP-1 or Exendin 4
7. Nicotinamide (Sigma)
8. Non-essential amino acid / NEAA (Invitrogen)
9. 0.25% trypsin (GIBCO, cat. no. 27250-018)

Equipment

1. Hood for cell culture with vertical laminar flow and equipped with UV light for decontamination (PC2 certified)
2. Water bath with temperature control
3. Clinical centrifuge
4. Incubator with both temperature and gas composition controls
5. Inverted microscope for cell culture
6. Disposable Pipettes (ARS, cat. no. 537-503/200)
7. T-75 culture flask

Reagent Setup

1. Media composition:

Day 0 (day of step-down):

DMEM / F12: Mixed 1:1 (or CMRL media for hiPCs)

DMEM was obtained from Gibco (Catalog # 11885-084)

F-12 medium was from Cellgro (Catalog # 10-080-CV)

BSA: 1 % (wt/v): Add 1g to 20 ml of media and keep it in water bath at 37°C for ~30-45 mins to dissolve. Do not shake / froth. Filter this using 0.22µm syringe filter.

ITS (available as 100X): add 1 ml to the media (for 100ml final media)

Mix all of the above, make volume to 100ml and check pH.

Day 4

Day 0 media

Taurine (Sigma catalog # T-8691; FW 125.1): 0.3mM

Day 10

Day 0 media* (everything else is same except BSA increased to 1.5%)

Taurine (Sigma catalog # T-8691; FW 125.1): 3.0mM

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GLP-1 / Exendin (Stock 100 μ M): 100 nM
Nicotinamide: 1mM
NEAA: 100 μ M

Procedure:

1. Take passage 3-7 PANC-1 cells \rightarrow remove SCM and add ~ 8ml Trypsin.
2. Incubate this at 37°C for 2-3 mins and tap the flasks to dislodge the cells. If using a plate, tap the sides gently and then resuspend the cells by pipetting the media gently.
Note: DO NOT add serum at any point to these cells once the complete media (containing FBS/FCS) is removed and the cells are exposed to trypsin
3. Resuspend the pellet in day 0 SFM and pipette cells using minimal pipette force for ~ 20 times
4. Plate the cells on to the Sigma-non adherent culture plates (helps to hasten the aggregation process) or simply use the BD cell culture treated flasks (for slower aggregation).
5. Feeding cells:
 - A considerable (~10-20%) cell death may be seen after 1 day of step-down; therefore re-feed the cells the next day (~18hrs) with day 0 SFM and then after every 2 days starting on 4th day with the day 4 SFM.
6. The day 10 SFM starts on day 10 (or earlier, as discussed) and the clusters are fed every other day. Do not spin the cells in a centrifuge at any point during differentiation; allow them to settle in the 15 ml conical tube and pull off the supernatant once the aggregates have settled down (usually in a minute or so). If you are doing this the first time, then take off the supernatant that you pull out into a petri plate and confirm that you have not lost any clusters.

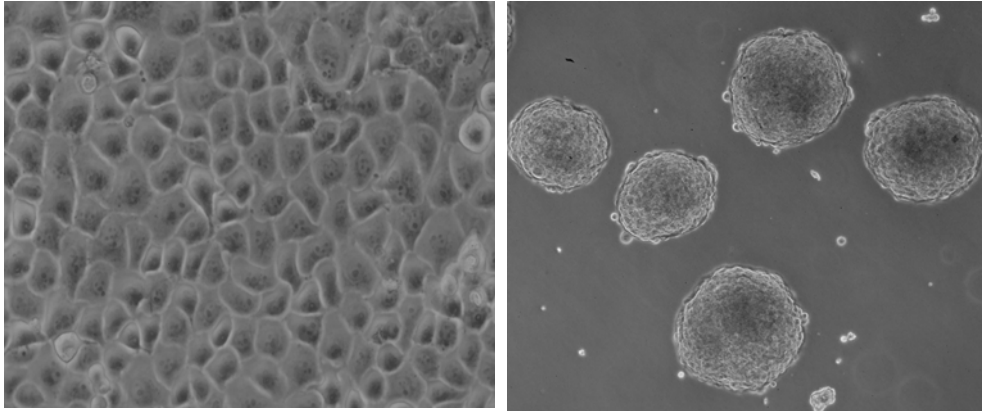
Anticipated results

Take 80-90% confluent flasks (T-75) for step-down protocol. The passage number obtained from ATCC was passage 57 and this will be referred to herein as passage 0. The cells that were first obtained from these are passage 1 and so on. In my experience, passages 3-7 have been the best candidates in achieving decent aggregation / differentiation of these precursor PANC-1 cells. Otherwise, use the re-cloned PANC1 cells.

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Representative image / picture

PANC1 cells at day 0 (left) and day 10 (right panel) after induction of differentiation.



References:

Wu Y, Li J, Saleem S, Yee S, Hardikar AA and Wang R (2010) C-Kit and stem cell factor regulate PANC-1 cell differentiation to insulin- and glucagon-producing cells. *[Lab Investigation](#)* (In press)

Gershengorn MC, Hardikar AA, Wei C, Geras-Raaka E, Marcus-Samuels B, Raaka BM (2004) Epithelial-to-Mesenchymal Transition Generates Proliferative Human Islet Precursor Cells. *[Science](#)* 306:2261-2264.

Hardikar AA, Marcus-Samuels B, Geras-Raaka E, Raaka BM, Gershengorn MC (2003) Human pancreatic precursor cells secrete FGF2 to stimulate clustering into hormone-expressing islet-like cell aggregates. *[Proc Natl Acad Sci U S A](#)*. 100(12): 7117-7122.