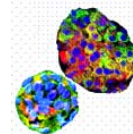


# SOP



Title:	<b>PANC1 cell maintenance</b>				
Protocol #:	1.2	Submitted:	050510	Approved:	200610
Category:	CC	Author(s): <sup>1</sup>	MRU, AAH	Checked by:	AAH

## Reagents:

1. DMEM (Cellgro catalog # 10-013-CV; 4.5g/L glucose)
2. FBS (GIBCO, cat. no. 10270-106)
3. 0.25% trypsin (GIBCO, cat. no. 27250-018)
4. L-glutamine (GIBCO, cat. no. 35050-038)
5. FBS (GIBCO, cat. no. 10270-106)

## 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 (no temperature control is needed)
4. Incubator with both temperature and gas composition controls
5. Inverted microscope with phase-contrast capabilities
6. Disposable Pipettes
7. Culture flasks

## Reagent Setup

Complete DMEM medium (with 10% FBS) stored at 4 °C should be kept in 37°C waterbath for ~ 30 mins to warm it before use.

Note: I generally prefer no antibiotics in the media, but these can be added if necessary. All antibiotics are stored in the freezers in each culture room.

## Procedure:

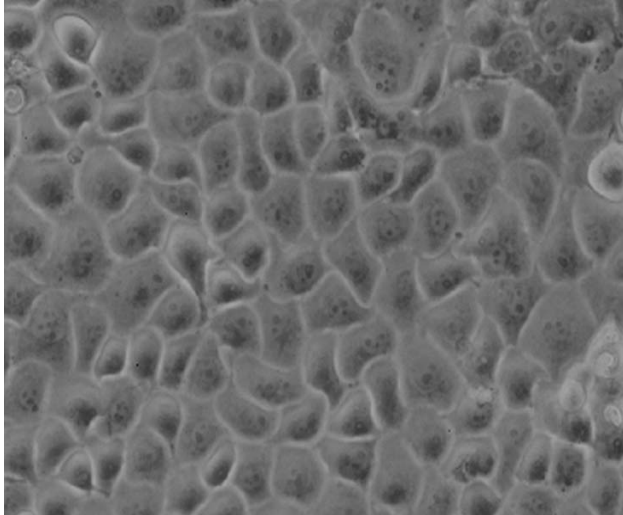
1. Remove all the media from confluent flasks using a 10 ml disposable pipette and add ~2-5 ml of trypsin (to 25cm<sup>2</sup> or 75cm<sup>2</sup> flask).
2. Incubate at 37°C for 2 – 3 mins
3. Tap the flasks and remove all the adherent cells by pipetting using a 10 ml pipette.
4. Centrifuge for 3 mins at 1200 rpm
5. Remove trypsin and resuspend cells in Complete DMEM media.
6. Split cells 1 → 3 (A confluent 75cm<sup>2</sup> flask makes 3 new 75cm<sup>2</sup> flasks)
7. Use 9 ml media in 75cm<sup>2</sup> flasks and ~3.5 to 4ml media for 25cm<sup>2</sup> flasks. If you are planning to use the cells for induction of differentiation to endocrine pancreatic lineage (“step-down protocol”) then DO NOT add serum containing media to these cells at any time after exposure to trypsin. Always split cells when they are ~ 80-90% confluent.

# SOP

## Anticipated results

If left unattended they tend to form some clusters on top of the monolayer of cells.

## Representative image / picture



A confluent monolayer of PANC1 cells in complete DMEM (serum-containing medium), prior to passaging.

## Notes:

PANC-1 cells were obtained from ATCC (CRL-1469). They were received as  $2.9 \times 10^6$  cells / ml, passage 57, lot # 1447840 (Another lot received was #1671194; same freeze date, no significant lot variation detected). The cell line was obtained from an epithelioid carcinoma of the pancreatic ducts of a 56 year old Caucasian male. However, passaging these for longer terms (additional 15-30 passages) introduced a lot of variability in expression of duct markers and we recloned this by clonal dilution and expansion.