Applied StemCell, Inc.
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Datasheet

Pig Induced Pluripotent Stem Cells (piPSCs, Episomal) Passage # 5

Order information

Catalog Number	Quantity
ASE-9106	2.0 x 10^6 cells/ vial

Product Information

The domesticated pig's (Sus scrofa) long lifespan, its similarities to humans in both organ physiology and morphology, its large body size and the advantages of being a relatively inexpensive and publically acceptable alternative to primates makes the pig an ideal animal model for the efficacy of iPSC based regeneration technologies (1-3). Applied StemCell Inc. is proud to provide Pig Induced Pluripotent Stem Cells (piPSCs) (Figure 1). These fully characterized, piPSCs can be further differentiated to the particular cell types of the investigator's choice, thus facilitating studies in cell replacement therapies and disease modeling.

Applied StemCell's piPSCs are low passage (p5) stem cells. Footprint-free pig iPS (induced pluripotent stem) cell line was derived from Duroc pig dermal fibroblasts by ectopic expression of OCT4, SOX2, KLF4, and L-MYC genes using episomal plasmids. The piPSCs were selected and expanded using morphological criteria (i.e. without the use of fluorescent marker or drug selection). When cultured under specific conditions (4), the pig iPS cells display ES-like morphologies with compact cell adhesion and clear colony borders (Figure 2). These piPSC colonies also express standard pluripotency markers (TRA-1-60, Oct4) and display strong alkaline phosphatase activity (Figure 3). These piPSCs are further characterized using RT-PCRs for the expression of an extended panel of pluripotency markers (Figure 4) in addition to those included in immuno-histochemistry (IHC) characterization.

Protocols for the culture, freezing and thawing of these piPSCs are provided.

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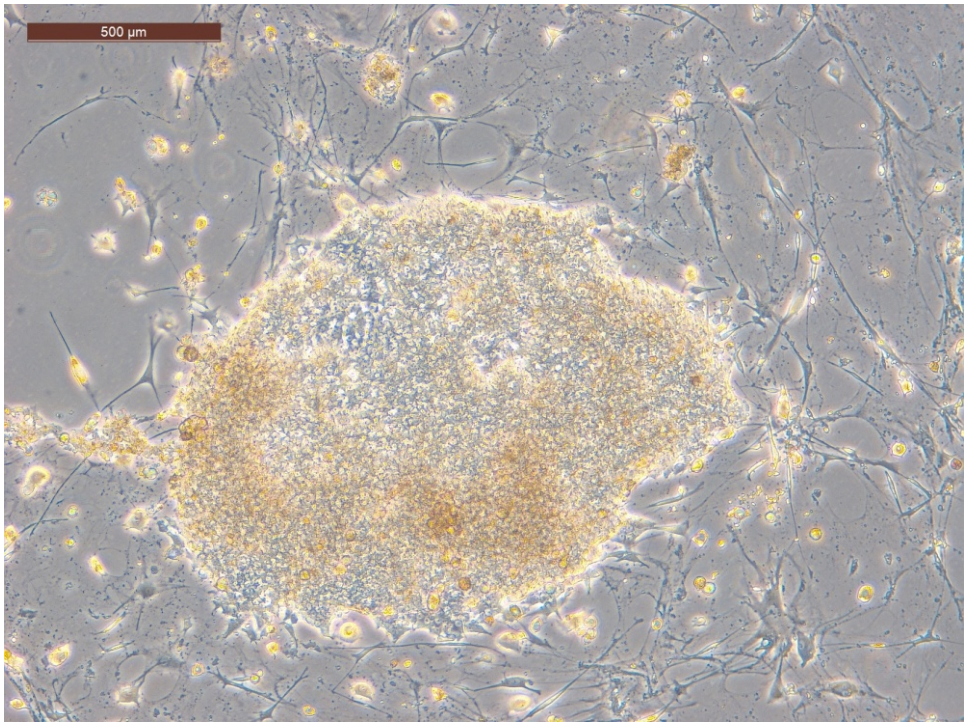


Figure 1. A representative presentation of a Pig Induced Pluripotent Stem Cell Colony cultured on mouse embryonic fibroblast (MEF) cells.

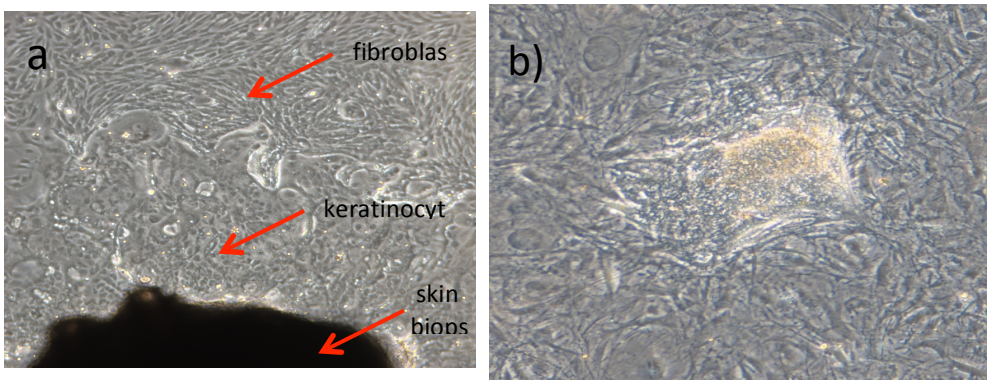


Figure 2. a) Duroc pig dermal fibroblasts (d16) emerging from a belly skin biopsy, and b) a piPSC colony.

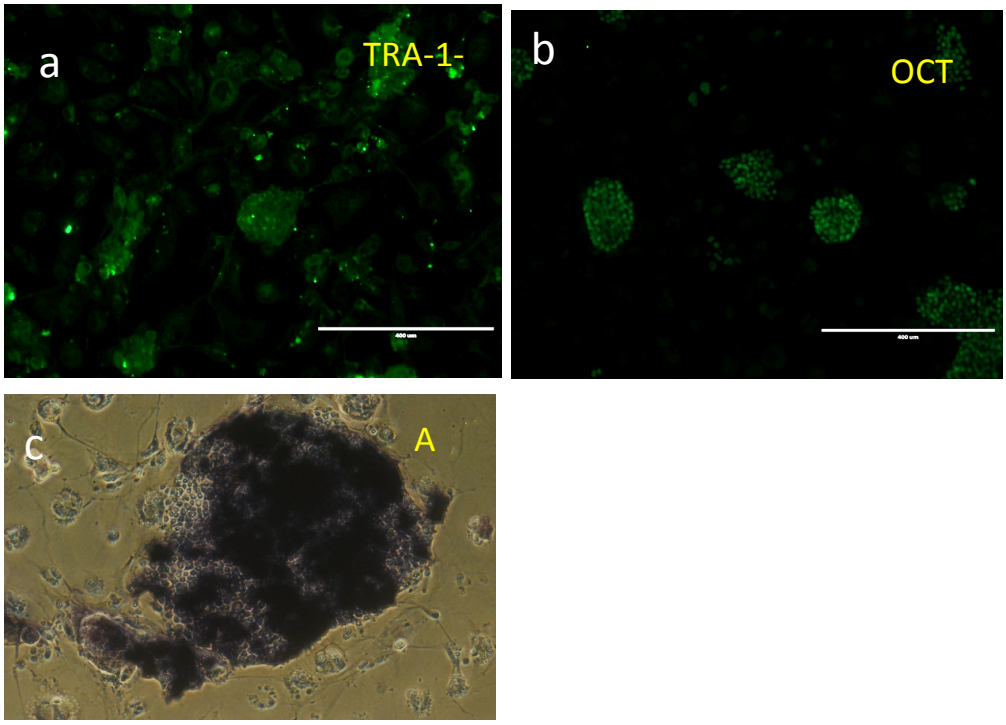
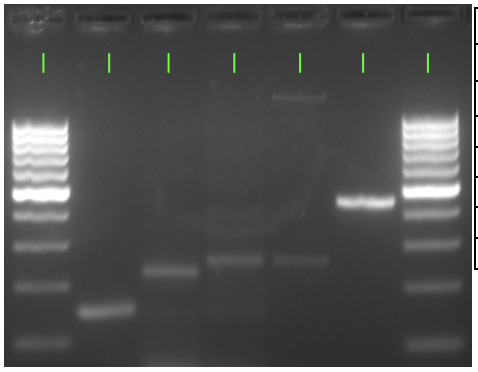


Figure 3. IHC characterization: Pig iPSC colonies stained with a) Tra-1-60, b) Oct-4, and c) Alkaline Phosphatase.



Lane	Gene	PCR prod (bp)
1	100 bp ladder	/
2	OCT-4	155
3	SOX-2	244
4	Nanog	255
5	hTERT	256
6	G3PDH (control)	452
7	100 bp ladder	/

Figure 4. RT-PCR Characterization: Total RNA isolated from Duroc pig iPSCs was used to generate cDNA. The following iPSC primer pair expression panel was then used to characterize the iPSC: Oct-4(155bp), Sox-2 (244 bp), Nanog (255bp) and pTERT (256bp).

The G3DPH primer pair (452 bp) was used as a positive control.

Quantity $2x10^6$ cells/vial

Passage P5

Shipping Dry ice

Storage and Stability Store the cells in vapor phase of liquid nitrogen immediately upon receipt. This product is stable for 6 months from the date of receiving when stored as directed.

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Media and Materials

Pig iPS Cell Culture

PROCEDURE

Reagents required but not provided:

1. Knockout DMEM/F12 (Cat.no. 12660-012, Invitrogen)
2. Knockout serum replacement (Cat.no. 10828-028, Invitrogen)
3. ES FBS (Cat.no. 16141-061, Invitrogen)
4. GlutaMAX (Cat.no. 35050-061, Invitrogen)
5. Nonessential amino acids (Cat.no. 11140-050, Invitrogen)
6. 2-mercaptoethanol (Cat.no. 21985-023, Invitrogen)
7. bFGF (Cat.no. 233-FB-025, R&D Systems or equivalent)
8. Human LIF (Cat.no. 300-05, Peprotech)
9. Penicillin and streptomycin (Cat.no. 15140-122, Invitrogen)
10. ROCK inhibitor (Y-27632, Cat.no. Y-05, StemRD)
11. CF1-MEF 6-well plates

Preparation of piPSC Media

Reagent	Final Conc.
Knockout DMEM/F12	/
Knockout Serum Replacement	10%
ES FBS	10%
GlutaMax	2 mM
NEAA	0.1 mM
2-mercaptoethanol	0.1 mM
FSF-2	10 ng/mL
hLIF	10 ng/mL
Pen/Strep	50 ug/mL

Recommended MEFs

Cat.#: ASF-1214 CF-1 MEFs
Cat.#: ASF-1014 DR4 MEFs

Protocol

Thawing/plating cryopreserved piPS cells

1. Before thawing the piPS cells, prepare a feeder cell plate (6-well plate) with MEFs.
2. Warm the piPSC medium to 37°C before use. Due to the low survival rate of cryopreserved piPS cells, the recovery is expected to take at least one week.
3. Quickly thaw the piPS cells in a 37°C waterbath by gently shaking the cryovial continuously till half thawed. Remove the cryovial from the waterbath and spray with 70% ethanol.
4. Transfer the contents of the cryovial to a 15 mL conical tube. Add 5 mL warm pig ES medium dropwise to the tube, mixing gently while adding medium.
5. Centrifuge cells at 200 x g for 5 minutes at room temperature.
6. While centrifuging, remove MEF medium from the feeder cell plates, and wash the wells twice with Knockout DMEM/F12. Then add 1 ml of piPS cell medium with 5 µM ROCK inhibitor Y-27632 (Cat.#: Y-05, StemRD) to one well of a 6-well plate.
7. Aspirate the medium from the 15 mL tube. Gently resuspend the cell pellet in 1 mL of fresh piPS cell medium containing 5 µM ROCK inhibitor Y-27632.
   NOTE: The viability of the piPSC will be maximized if they are maintained as small cell clumps.
8. Transfer the medium containing the piPSC clumps to the well on the 6-well plate with MEF feeder cells.
9. Rock the plate gently to distribute the clumps evenly in the wells. Incubate the cells at 37^0C/5%CO2 overnight.
10. Change medium daily. Check for undifferentiated colonies that are ready to passage when colonies are big enough.

Plating/Passaging piPSC colonies

1. Aspirate the medium and wash the cells twice with 1 ml of PBS.
2. FOR ENZYMATIC PASSAGING: Remove PBS completely and add 0.5 ml of 0.25% Trypsin (Cat.#: 25200-056, Invitrogen) and incubate for 1-2 min at room temperature.
3. Add 1 ml of piPSC medium to the plate and suspend the cell colonies by pipetting up and down to harvest piPS cells.
4. Remove a plate of MEF feeder cells from the incubator. Aspirate the MEF medium. Wash once with KO DMEM/F12 medium.
5. Distribute 0.1 – 0.2 ml of the pig iPS cell suspension to each well of a 6-well plate. Add piPSC media to a final volume of 2 ml per well. Right after plating the iPS cells, gently rock the plate and incubate at 37°C.
6. Next day, replace the media with fresh piPSC media.
7. FOR MANUAL PASSAGING: Place an inverted microscope in a tissue culture hood and prepare the MEF plates per step d.
8. Manually dissect the piPSC colonies.
9. Collect the colony pieces using a p200 Pipetman^R and seed these pieces on the MEF plate.
10. The piPSC medium must be changed every day and piPS cells subcultured every 4-7 days. Track the passage number of the cells.

Cryopreserving pig iPS cells

1. Prepare EZStem freezing medium (Cat.#: M050, ALSTEM) or piPSC media with 10% DMSO on ice.
2. FOR ENZYMATIC RETRIVAL OF THE COLONIES: Aspirate the media and wash the cells twice with 1 ml of PBS.
3. Remove PBS completely and add 0.5 ml of 0.25% Trypsin (Cat.#: 25200-056, Invitrogen) and incubate for 1-2 min at r room temperature.
4. Add 2 ml of piPSC media to the plate and suspend the cell colonies by pipetting up and down to harvest piPS cells.
5. Transfer the contents to a 15 mL conical tube. Centrifuge cells at 200 x g for 5 minutes at room temperature.
6. After centrifugation, aspirate the medium from 15 mL tube. Gently resuspend the pellet in 5 ml of freezing medium.
7. Transfer 1 mL of cell suspension in freezing media into each labeled cryogenic vial.
8. Place vials into an isopropanol freezing container and place the container at -80°C overnight.
9. Transfer to a liquid nitrogen tank next day.
10. FOR MANUAL RETRIEVAL OF THE COLONIES: Place an inverted microscope in a tissue culture hood and prepare the freezing media per step a.
11. Manually remove the piPSC colonies from the MEF plate.
12. Suspend the cell colonies by pipetting up and down and proceed to steps d-i.

References

1. Roberts, R.M., et al. Induced Pluripotent Stem Cells from Swine (Sus scrofa) Cell Cycle 8(19):3078-81 (2009).
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3. Zhou, L., et al. Differentiation of Induced Pluripotent Stem Cells o f Swine into Rod Photoreceptors and Their Integration Into the Retina. Stem Cells. 29:972-980 (2011).
4. Fujishiro, S., et al. Generation of Naïve-Like Porcine-Induced Pluripotent Stem Cells Capable of Contributing to Embryonic and Fetal Development. Stem Cells and Development 22(3): 4733–482 (2013).