Applied StemCell, Inc.
(866) 497-4180
www.appliedstemcell.com

Datasheet

Guinea Pig Induced Pluripotent Stem Cells (gpiPSCs)

Order information

Catalog Number	Quantity
ASE-9108	1.0 x 10^6 cells/ vial

Product Information

Strain Hartley

Gender Male

Passage P7

Description & Background Information

Guinea pigs have been used as research models for several centuries (1). They are primarily used as models for human medical conditions such as infectious diseases (e.g. tuberculosis (2)), juvenile diabetes, as well as pre-eclampsia and other reproductive studies (3).

Applied StemCell's guinea pig induced Pluripotent Stem Cells (gpiPSCs) were generated from guinea pig fibroblast using retroviral reprogramming plasmids (4, 5; Fig.1). These pluripotent gpiPSCs can be further differentiated to numerous cell types, thus facilitating studies in cell replacement therapies and disease modeling.

When cultured according to protocol, the gpiPS cells display embryonic stem cell (ESC)-like morphology with compact cell adhesion and distinct colony borders. The colonies express standard pluripotency markers (e.g. Oct-4, Nanog, and SSEA-1) and display strong alkaline phosphatase activity. The gpiPSCs are further characterized by RT-PCR for the expression of an extended panel of pluripotency markers as well as for teratoma analysis.

Shipping Dry ice.

Storage and Stability Store the cells in vapor phase of liquid nitrogen immediately upon receipt. This product is stable for at least 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.

Applied StemCell, Inc.
521 Cottonwood Dr. #111, Milpitas, CA 95035
Phone: 866-497-4180 (US Toll Free); 408-773-8007 Fax: 408-773-8238
info@appliedstemcell.com www.appliedstemcell.com

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

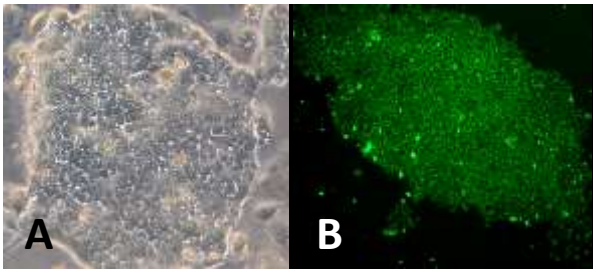

Figure 1. (A) Established gpiPSC line. (B) giPSC colony stains positive for pluripotency marker Oct-4.

Media and Materials

Reagent Formulas

gpiPSC Culture Medium

(Store prepared medium at 4°C for up to one week)

Component	Final Concentration
DMEM/F12	
Fetal bovine serum (FBS)	*15%*
L-Glutamine	2 mM
Nonessential amino acids	0.1 mM
2-Mercaptoethanol	0.1 mM
Leukemia inhibitory factor (mouse)	10 ng/ml
Penicillin and streptomycin (optional)	50 ug/ml

gpiPSC Freezing Medium

Component	Final Concentration
gpiPSC Culture Medium	90%
DMSO	10%

Other Reagents required but not provided

- Mouse embryonic fibroblast (MEF) cells; #ASF-1214 (CF1); Applied StemCell
- Trypsin-EDTA (0.05%)

Protocol

Thawing/plating cryopreserved gpiPS cells

1. Before thawing the gpiPS cells, prepare a cell culture plate (6-well plate) with MEF cells.
2. Quickly thaw the gpiPS cells in a 37°C water bath by gently shaking the cryovial until half thawed.
3. Transfer the contents of the cryovial to a 15 ml conical tube. Add 5 mL gpiPSC culture medium dropwise to the tube and mixing gently.
4. Centrifuge cells at 200 x g for 5 minutes.
5. Aspirate the MEF medium from the feeder cell plates, and wash the wells with Knockout DMEM/F12. Add 1 mL of gpiPSC culture medium to one well of a 6-well plate.
6. Aspirate the medium from the 15 mL tube and gently resuspend the cell pellet in 1 ml of fresh gpiPSC culture medium.

   NOTE: The viability of the gpiPSC will be maximized if they are maintained as small cell clumps.

7. Transfer the medium containing the gpiPSC clumps to the well on the 6-well plate with MEF feeder cells.
8. Rock the plate gently to distribute the clumps evenly and incubate the cells at 37^0C / 5% CO_2.
9. Change medium daily. Check for undifferentiated colonies that are ready to passage when colonies are big enough.

Passaging gpiPSC colonies

10. Prepare a fresh MEF-covered cell culture plate the day before passaging.

    NOTE: We recommend passaging gpiPS cells manually, but enzymatic passaging is also possible.

11. FOR MANUAL PASSAGING: Prepare the MEF plates as described in Step 5.
12. Aspirate the gpiPSC medium and wash the gpiPS cells with 1 ml of PBS.
13. Manually dissect the piPSC colonies using an inverted microscope in a tissue culture hood.
14. Collect the colony pieces using a p200 Pipetman^R and seed these pieces on the MEF plate at a ratio of 1:3 – 1:6, depending on the density.
15. Continue with Step 21.
16. FOR ENZYMATIC PASSAGING: Prepare the MEF plates as described in Step 5
17. Aspirate the gpiPSC medium and wash the gpiPS cells with 1 ml of PBS.
18. Add 0.25% Trypsin and incubate for 1-2 min at room temperature.
19. Add 1 ml of gpiPSC culture medium to the trypsin and suspend the cell colonies by pipetting up and down.
20. Distribute the gpiPS cell suspension to each well of a 6-well plate at a ratio of 1:3 – 1:6, depending on the density.
21. Add gpiPSC media to a final volume of 2 ml per well and gently rock the plate before placing it back into the incubator.
22. Next day, replace the media with fresh gpiPSC media.
23. The gpiPSC medium must be changed every day and gpiPS cells subcultured every 4-7 days.

Cryopreserving gpiPS cells

24. Prepare gpiPSC freezing medium with 10% DMSO on ice.
25. FOR MANUAL RETRIEVAL OF THE COLONIES: Place an inverted microscope in a tissue culture hood. Aspirate the media and wash the cells with 1 ml of PBS.
26. Manually remove the gpiPSC colonies from the plate.
27. Suspend the cell colonies by pipetting up and down and proceed to Step 31.
28. FOR ENZYMATIC RETRIEVAL OF THE COLONIES: Aspirate the medium and wash the cells with 1 ml of PBS..
29. Add 0.5 ml of 0.25% Trypsin and incubate for 1-2 min at room temperature.
30. Add 2 ml of gpiPSC media to the plate and suspend the cell colonies by pipetting up and down.
31. Transfer the contents to a 15 ml conical tube. Centrifuge cells at 200 x g for 5 minutes.
32. Aspirate the medium from 15 mL tube. Gently resuspend the pellet in 5 ml of freezing medium.
33. Transfer 1 mL of cell suspension in freezing media into each labeled cryogenic vial.
34. Place vials into an isopropanol freezing container and place the container at -80°C overnight.
35. Transfer to a liquid nitrogen tank next day.

References

1. M.E. Reid (1958). *The Guinea Pig in Research*. Human Factors Research Bureau. pp. 62–70.
2. S. Clark et al. (2014). *Animal Models of Tuberculosis: Guinea Pigs.* Cold Spring Harb Perspect Med. doi: 10.1101/cshperspect.a018572.
3. D.H. Percy et al. (2001). *Pathology of Laboratory Rodents and Rabbits* (2nd ed.). Iowa State University Press. pp. 209–247.
4. K. Takahashi et al. (2007) Cell. 131, 861-72.
5. G. Nagamatsu et al. (2012) J Biol Chem. 287, 36273-82.