Applied StemCell, Inc.
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Datasheet

Human Induced Pluripotent Stem Cells

Product Information

Catalog Number **ASE-9202**

Description Applied StemCell, Inc. provides Induced Pluripotent Stem (iPS) cells with low passages (p5-p7). These pluripotent cells were generated from human skin fibroblast using a non-integrating, footprint-free reprogramming method (1). This method results in the transient expression of human transcription factors (OCT4, SOX2, KLF4, and c-MYC) that initiate the reprogramming process (2). The resulting iPS cells were selected using morphological criteria without the use of either fluorescent markers or drug selection. These iPS cells have been tested for the expression of the pluripotency markers, including Oct4, Sox2, SSEA4, and TRA-1-60, AP (alkaline phosphatase) activity and for functional pluripotency via teratoma formation analysis. Detailed protocols for thawing, culture, on both feeder and feeder-free conditions, and freezing of these iPS cells are provided.

Quantity 2-5x10^5 cells/vial

Shipping Dry ice

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

Quality Control Each lot of human iPS cells has been tested for growth and viability following recovery from cryopreservation. In addition, each lot has been tested for functional pluripotency (via teratoma formation in mice), the expression of stem cell markers (Oct4, Sox2, SSEA4 and TRA-1-60) and for the absence of mycoplasma.

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.

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Media and Material

iPSC Culture Medium Formula (for feeder-dependent culture system)

Material	Vendor	Cat. Number
Culture medium for human ES/iPS cells		
DMEM/F12 medium	Applied StemCell	ASM-5002
MEF cells	Applied StemCell	ASF-1214/1216 or ASF-1224/1226
FBS	Applied StemCell	ASM-5017/5007
100X Non-essential amino acids	ThermoFisher	1114005
100mM Na-Pyruvate	ThermoFisher	11360070
100X Pen/ Strep Solution	ThermoFisher	15140122
FGF-2	Peproptech	AF-100-18B
StemPro® EZPassage™ Disposable Stem Cell Passaging Tool	ThermoFisher	23181010
Gelatin		
Hemocytometer		

Cryopreserving Human iPS cells

Material	Details
2x Human ESC Freezing medium	Kept on ice
Culture medium	Kept on ice
One 6-well plate of iPS cells ready to be passaged	
Labeled cryovials on ice	
Isopropanol freezing jar	

iPSC Culture Medium Formula (for feeder-free culture system)

Material	Vendor	Cat. Number
DMEM/F12 medium	Applied StemCell	ASM-5002
Serum/Feeder Free Medium (SFFM)	Applied StemCell	ASM-5010
Geltrex®	ThermoFisher	12760-021

Protocol

Feeder-dependent culture conditions

1. Preparation of CF-1 MEF feeder plates *(for feeder-dependent culture conditions)*

1.1. Coat each well of a 6-well plate with 1mL of 0.2% sterile gelatin. Let it stand at room temperature for a minimum of 30 minutes. Aspirate the gelatin and add 2 ml of MEF complete media (DMEM medium, 10% FBS, 1x glutamine, 1X Na Pyruvate, 1X nonessential amino acids, 1X Pen/Strep) to each well.
1.2. Using a 15mL conical tube, warm 9mL of MEF complete media for 10 minutes at 370C.
1.3. Quickly thaw one vial of CF-1 MEFs (ASC cat# ASF-1214/1216 or ASF-1224/1226) in a 37°C water bath. Remove the cryovial from the water bath and spray with 70% ethanol.
Note: Be sure to exercise the required safety precautions outlined above.
1.4. Transfer the contents of the cryovial to the 15 mL conical tube. Mix gently. Remove 10 µL and add to one chamber of a hemocytometer.
1.5. Count the cells in the chamber and determine the cell density (#cells/ mL) in the 15mL conical tube.
1.6. Calculate the volume needed for 2.5x10^5 cells/well. Add this volume to each well of the 6-well plate.

2. Preparation of human iPS cell culture medium *(feeder dependent)*

2.1 Thaw 100 mL of culture medium at room temperature or at 4^0C overnight.
2.2 Add 1 mL of stock 100X Pen/Strep solution.
2.3 Add 200µl of a 10µg/mL FGF-2 stock solution (20ng/ml final).

3. Thawing/plating cryopreserved human iPS cells

Note: Due to the inherent low survival rate of cryopreserved human iPS cells, be sure to warm the media to 37°C before use. The recovery of frozen iPS cells could take at least one to two weeks.

3.1. Prepare a MEF-covered 6-well plate one day before the scheduled thawing (see 1.).
3.2. Warm a 20 ml aliquot of iPSC culture medium for 30 minutes at 37°C.
3.3. Quickly thaw the human iPS cells in a 37°C water bath. Remove the cryovial from the water bath and spray with 70% ethanol.
3.4. Transfer the contents of the cryovial to a 15 mL conical tube containing 5 mL of warm iPSC culture medium, mixing gently to not disturb the iPS cell colony chunks.
3.5. Immediately centrifuge the cells at 200x g for 5 minutes at room temperature.
3.6. Carefully aspirate the medium and resuspend the pellet in 2 mL of warm iPSC culture medium. Again, mix gently to not disrupt the iPS cell colony chunks.
3.7. Aspirate the media from the MEF-covered well. Wash once with 1X PBS.
3.8. Plate iPS cells evenly in each well of the MEF-covered 6-well plate. Distribute the iPS cell chunks by gently rocking the plate in cross pattern.
3.9. Place the plate in a 37°C incubator and rock the plate one more time to evenly distribute the iPS cell clumps in the well. Culture the cells at 37°C and 5% CO_2.
3.10. Change the iPSC culture medium daily and monitor the cells.
3.11. The iPS cell colonies should be big enough to passage approximately 7-10 days after plating.

Notes:
- *Due to the inherent low survival rate of cryopreserved human iPS cells, be sure to warm the media to 37°C before use. The recovery of frozen iPS cells could take at least 1-2 weeks.*
- *It is recommended that the frozen iPS cells be initially plated on a feeder surface. Once the line is established, cells can easily be transitioned to feeder free-culture condition.*

4. Passaging human iPS cells

4.1. Aspirate the medium from the plate and wash once with sterile 1X PBS.

4.2. (Optional, but suggested) Add 1 ml Collagenase IV (1 mg/ml) to the plate and incubate at 37ºC for 15 min.
4.3. Add 2 ml of iPSC culture medium (when using MEFs) or supplemented SFFM (when using feeder-free system) per well.
4.4. Use the StemPro® EZPassage™ Disposable Stem Cell Passaging Tool per the manufacturer's instructions.
4.5. Gently remove the iPS cell colony pieces from the well surface using the cell passaging tool.
4.6. Transfer the iPS cell colony pieces into a 50 mL conical centrifuge tube.
4.7. Wash the well 1-2 times with iPSC culture medium/supplemented SFFM
4.8. Add additional medium to the tube such that your total volume is ~15 mL
4.9. Allow the iPS cell colony pieces to settle by gravity for ~2-3 minutes or until the overly large chunks settle to the bottom of the tube.
4.10. Remove the supernatant (containing optimally sized colony pieces) using a serological pipette. Be careful not to disturb the larger colony pieces that have collected on the bottom of the tube.
4.11. Evenly distribute (~2 ml/well) the colony pieces into the wells of a MEF- or Geltrex®-covered 6-well plate (remove MEF medium/Geltrex® before use).
4.12. Rock the plate in a back and forth cross pattern to evenly distribute the colony pieces.
4.13. Incubate the plate at 37ºC and 5% CO_2. Do not disturb the plate for 24 hours.
4.14. Once the colony pieces have attached, change the media daily until the colonies are ready for passaging.

Note: Once the colonies are at an optimal density for passaging, the cells can be split every 4-5 days at a 1:4 ratio.

5. Cryopreserving human iPS cells

5.1. Perform steps 4.1 - 4.9 for each well in the 6-well plate.
5.2. Remove the supernatant (containing optimally sized colony pieces) using a serological pipette and transfer to a fresh 15 ml conical tube. Be careful not to disturb the larger colony pieces that have collected on the bottom of the tube.
5.3. Centrifuge at 200x g for 3 minutes.
5.4. Carefully aspirate the supernatant.
5.5. Gently resuspend the pellet in 3 mL of ice-cold iPSC culture medium.
5.6. Gently add the same volume of 2x human ESC freezing medium to the cell suspension and mix well.
5.7. Add 1 mL of the iPS cell suspension to each cryovial.
5.8. Cap the cryovials tightly, place the vials in an isopropanol freezing container and immediately transfer container to -80ºC.
5.9. Store the freezing container at -80˚C overnight.
5.10. After 24 hours, transfer the frozen cryovials to a liquid nitrogen tank for long term storage.

Feeder-free culture conditions

1. Preparation of feeder-free medium

1.1 Thaw 100 mL of Serum/Feeder Free medium (SFFM) at room temperature or at 4^{0}C overnight.
1.2 Add 1 mL of stock 100X Pen/Strep solution.
1.3 Add 200µl of a 10ug/mL FGF-2 stock solution (20ng/µl final concentration).

2. Coating plates with Geltrex® (*for feeder-free culture conditions*)

Note: Geltrex™ should be aliquoted (e.g. 0.25 mL in a 15 ml conical tube) and stored at -80°C until ready to use.

2.1 Thaw a 0.25 mL aliquot of Geltrex® completely on ice. Add 7.25 mL of cold (4ºC) DMEM/F12 medium and mix thoroughly.
2.2 Immediately coat the tissue culture plate with the diluted Geltrex® solution. For a 6-well plate, use 0.8-1.0 mL of diluted Geltrex® solution per well. In order to evenly distribute the Geltrex™ over the well surface, rock the plate back and forth in a cross pattern.

2.3 Let the coated plate stand for 1 hr at 37°C or overnight at 4°C (seal the plate with Parafilm if you decide to incubate overnight at 4ºC). If the plate has been stored at 4°C, incubate the plate at 37°C for at least 60 minutes before using.

Note: Due to the inherent low survival rate of cryopreserved iPS cells, be sure to warm the media to 37°C before use. The recovery of frozen iPS cells can take one-two weeks.

3. Thawing/plating cryopreserved human iPS cells

3.1 One hour before thawing the human iPS cells, warm the Geltrex® coated 6 well plate for one hour at 37°C.
3.2 Warm a 20 ml aliquot of SFF medium containing 20ng/mL FGF-2, 1X Pen/Strep for 30 minutes at 37°C.
3.3 Quickly thaw the human iPS cells in a 37°C water bath. Remove the cryovial from the water bath and spray with 70% ethanol.

Note: be sure to exercise the required safety precautions outlined above

3.4 Transfer the contents of the cryovial to a 15 mL conical tube. Immediately add 5 mL of warm SFF media to the tube. Mix gently and be careful not to disturb the iPS cell colony chunks.
3.5 Immediately centrifuge the cells at 3,000rpm for 3 minutes at room temperature.
3.6 Carefully aspirate the medium and resuspend the pellet in 2 mL of SFF media. Again, mix gently and be careful not to disrupt the iPS cell colony chunks.
3.7 Aspirate the Geltrex® solution from the 6-well plate. Wash once with sterile 1X PBS.
3.8 Plate the SFF media/iPS cell mixture in one well of the 6-well plate. Distribute the iPS cell chunks evenly by gently rocking the plate in cross pattern.
3.9 Place the plate in a 37°C incubator and rock the plate one more to evenly distribute the iPS cell clumps in the well. Culture the cells at 37°C, with 5% CO2.
3.10 Change the SFF media daily.
3.11 Monitor the well daily and manually remove any undifferentiated colonies.
3.12 The iPS cell colonies should be big enough to passage approximately 7-10 days after plating.

4. Passaging human iPS cells under feeder-dependent conditions

Follow the steps in section I-D

5. Cryopreserving human iPS cells

Follow the steps in section I-E.

References

1. Gonzalez, F., Boue, S., Belmonte, J. C. I., Methods for making induced pluripotent stem cells: reprogramming a la carte. (2011) *Nature Reviews/Genetics*, 12:232-42.

2. Takahashi, K., Tanabe, K., Ohnuki, M., Nanta, M., Ichisaka, T., Tomoda, K. and Yamanaka, S. Indution of pluripotent stem cells from adult human fibroblasts by defined factors. (2007) *Cell*. 131(5):861-72