Applied StemCell, Inc.
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Datasheet

Human Induced Pluripotent Stem Cells

Product Information

Catalog Number	**ASE-9203**
Description	Applied StemCell, Inc. provides Induced Pluripotent Stem (iPS) cells at low passages (p12). These pluripotent cells were generated from normal human skin fibroblast using a non-integrating, footprint-free reprogramming method (1). This method allows the transient expression of human transcription factors (OCT4, SOX2, KLF4, and c-MYC) that initiate the reprogramming process (2). The resulting human iPS cells (hiPSCs) 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, culturing under both feeder and feeder-free conditions, and cryopreservation of these iPS cells are provided.
Tissue	Dermal skin (fibroblasts)
Age	45 years
Sex	Male
Race	Caucasian
Clinical information	Normal
Quantity	2 – 5 x 10^5 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 expression of stem cell markers (OCT4, SOX2, SSEA4, TRA-1-81 and TRA-1-60 (Figure 1), functional pluripotency (via teratoma formation in mice; Figure 2), normal male karyotype (Figure 3), and for the absence of mycoplasma and pathogens (CoA available upon request).
Safety Precaution	**PLEASE READ BEFORE HANDLING ANY FROZEN VIALS**. Please wear appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling frozen vials. 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.

Characterization of iPSC Line ASE-9203

Pluripotency Marker Analysis

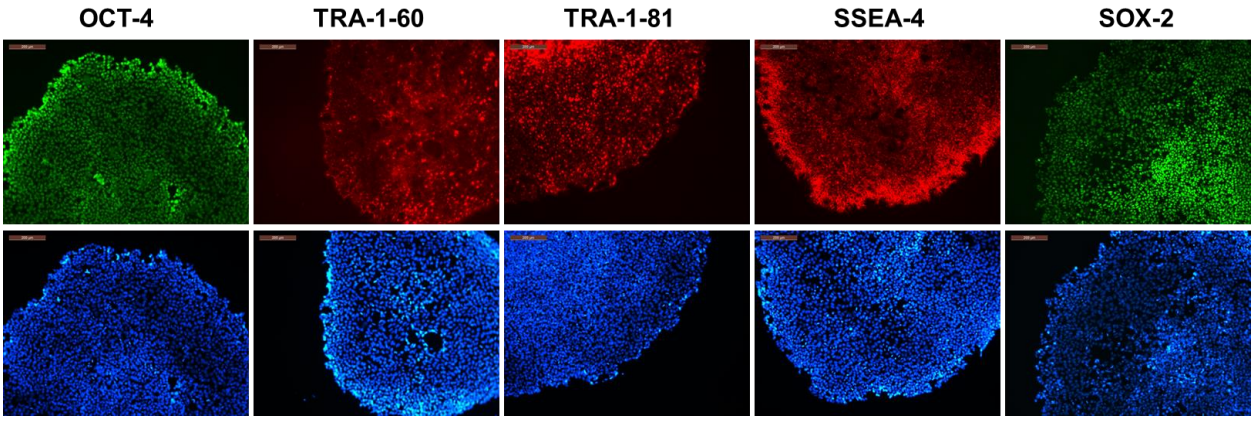
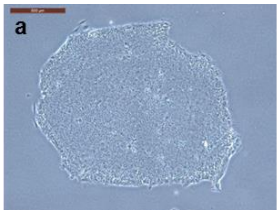


Figure 1a. Expression of pluripotency markers. ASC 9203 iPS cell line expresses common iPSC biomarkers (OCT-4, TRA-1-60, TRA-1-81 SSEA-4 and SOX-2). The corresponding DAPI staining is below each image. All images were taken at 10x magnification.



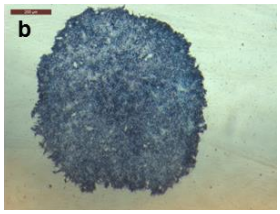
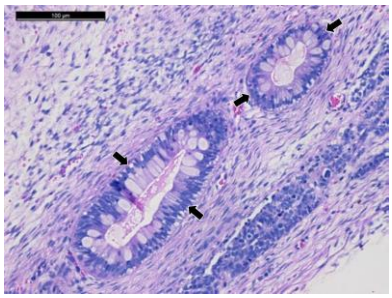
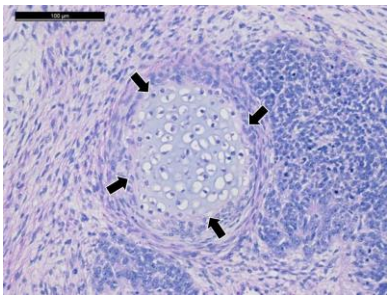


Figure 1b. Alkaline Phosphatase (AP) staining. ASC-9203 iPSCs stain positive for Alkaline Phosphatase: a typical unstained colony (a) was used to gauge the extent of the AP staining (b). Both images were taken at 5x magnification.

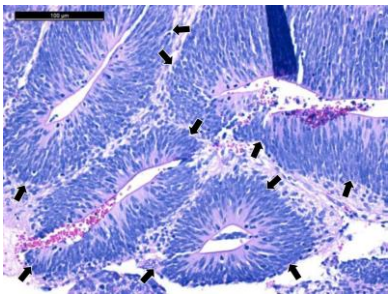
Teratoma Formation for Pluripotency Analysis



EN: Gland (200x)



ME: Cartilage (200x)



EC: Neuronal Rosette (200x)

Figure 1. Histological analyses using H&E staining of kidney and testis teratomas from mice injected with the ASE-9203 iPSC line. Differentiated tissues representing the three germ layers are shown and indicated by arrow heads. Abbreviations: EN: endoderm; ME: mesoderm; EC: ectoderm.

Karyotype Analysis

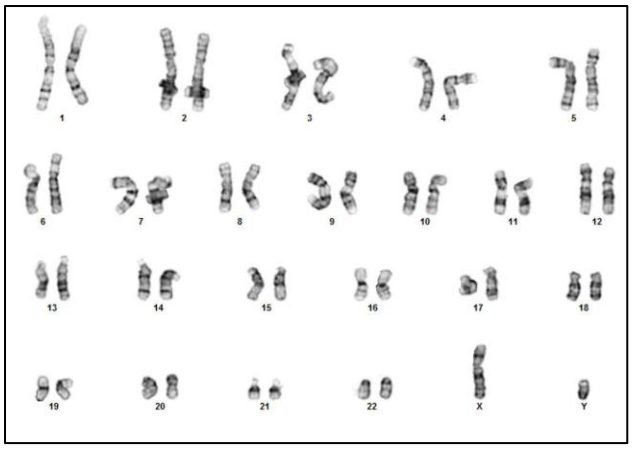


Figure 3. Karyotype analysis to rule out genetic aberrations. Cytogenic analysis was performed on G-banded metaphase cells from human iPSC line, ASE-9203. This iPSC line demonstrates a normal male karyotype.

Media and Material Required but not Provided

iPSC Media and Materials for Feeder-free Culture System

- TeSR-E8 medium, StemCell Technologies, Cat# 05940
- Rock Inhibitor, Stemgent, Cat# ASE-04-0012
- BD Matrigel™ hESC-qualified Matrix Features, BD Biosciences, Cat# 354277; or any synthetic basement matrix
- ES-Sure™ FBS, Applied StemCell, ASM-5017
- 1X PBS, ThermoFisher, Cat# 14190144
- 0.5 M EDTA, ThermoFisher, Cat# 15575020
- DMSO, Sigma-Aldrich, Cat# D8418
- Cell Scraper, Corning, Cat# 3010

iPSC Media and Materials for Feeder-dependent Culture System

- Culture medium for human ES cells (hESC)- refer to Section 2 of Feeder-dependent Culture Conditions
- DMEM/F12 medium; Applied StemCell, Cat# ASM-5002
- ES-Sure™ FBS, Applied StemCell, Cat# ASM-5017
- MEF Cells, Applied StemCell, Cat#s ASF-1244/ 1216/ 1224/ 1226
- 100X Non-essential amino acids, ThermoFisher, Cat# 11140050
- 100mM Na-Pyruvate, ThermoFisher, Cat# 11360070
- 100X Pen/ Strep Solution, ThermoFisher, Cat# 15140122
- 100X L-Glutamine, Thermofisher, Cat# 25030081
- b-FGF, R&D systems, Cat# 233-FB
- KnockOut Serum Replacement, ThermoFisher, Cat# 10828028
- Primocin, Invivogen, Cat# Ant-pm-2
- Rock Inhibitor, Stemgent, Cat# 04-0012-02
- 1X PBS, ThermoFisher, Cat# 14190144
- 0.5 M EDTA, ThermoFisher, Cat# 15575020
- DMSO, Sigma-Aldrich, Cat# D8418
- 55 mM 2-Mercaptoethanol (beta-Mercaptoethanol), ThermoFisher, Cat# 21985023
- Cell scraper, Corning, Cat# 3010
- StemPro® EZPassage™ Disposable Stem Cell Passaging Tool, ThermoFisher, Cat# 23181010
- Hemocytometer

Protocol

Feeder-free culture conditions

1. Thawing human iPSCs using a feeder-free protocol

1.1 Prepare 2.5 mL of TeSR-E8 medium + 10 µM Rock Inhibitor.
1.2 Aspirate the medium from a well of a 6-well plate; wash once with 1x PBS, and add 1 mL of TeSR-E8 medium + Rock Inhibitor. Label the plate with the name of the clone, passage number and date, and place it in the incubator.
1.3 Bring the cryovial on dry ice to the tissue culture room.
1.4 Quickly thaw the hiPSCs in a 37°C water bath by gently shaking the cryovial continuously until only a small frozen pellet remains.
1.5 Wipe the cryovial with a Kimwipe sprayed with 70% ethanol and place it into a biosafety cabinet.
1.6 Transfer the cells to a 50 mL conical tube.
1.7 Add 5 mL of cold TeSR-E8 medium dropwise while swirling the conical tube with the cells.
1.8 Centrifuge the cells for 5 min at 300g at 4°C.
1.9 Aspirate off the medium and add 1mL of TeSR-E8 medium + Rock Inhibitor.
1.10 Gently flick the conical tube to resuspend the cells and transfer them to the well of a Matrigel™ plate using a 5 mL serological pipette.
Note: Prepare Matrigel plates the previous day and not more than 3 days prior to thawing.
1.11 Place the plate in the incubator and move the plate back and forth and side to side, twice to spread the clumps evenly in the well.
1.12 Change medium daily. Usually, after 1-2 weeks the cells are ready to be split.

2. Passaging/ splitting human iPSCs using EDTA

2.1 Aspirate the medium from the hiPSC culture.
2.2 Wash once with 2 mL of 1x PBS.
2.3 Add 1 mL of 0.5 mM EDTA (in PBS) per well of a 6-well plate and incubate the cells for 3-5 min at RT.
2.4 Observe the cells under a microscope. When the cells at the edge of the colonies start separating and round up, aspirate the EDTA and add 1 mL of TeSR-E8 medium (or any medium for feeder-free culture of hiPSCs).
2.5 Scrape the cells from the bottom of the well until the colonies are floating; pipette up and down 2-3 times (with the 1000 µL pipette set to 800 µL) to break the colonies in small clumps.
Note: Pipetting up and down three times is enough to break the colonies into clumps of optimal size.
2.6 Transfer the desired dilution to the wells of the new Matrigel™-coated plate (usually at 1:3 – 1:10 ratio).
Note: Prepare Matrigel plates (or any other basement matrix) not more than a week prior to passaging cells
2.7 Place the plate in the incubator and move the plate back and forth and side to side, twice to spread the clumps evenly in the well.
Note: hiPSCs are passaged as clumps of 50-200 cells, rather than single cells. On average, cells need to be passaged once a week before the colonies are large enough to merge with one another.

3. Cryopreserving human iPS cells

3.1 Label the cryovials as needed, based on1 vial per well of a 6-well plate, and pre-chill them in a – 20°C freezer.
3.2 Aspirate the medium from the hiPSC culture.
3.3 Wash once with 2 mL of 1x PBS.
3.4 Add 1 mL of 0.5 mM EDTA (in PBS) per well of a 6-well plate and incubate the cells for 3-5 min at RT.
3.5 Observe the cells under a microscope. When the cells at the edge of the colonies start separating and round up, aspirate the EDTA and add 0.5 mL of cold TeSR-E8 medium.
3.6 Scrape the cells from the bottom of the well until the colonies are all floating.
Note: Do not pipette up and down when freezing hiPSCs.
3.7 Add 0.5 mL of cold 2x freezing medium dropwise while mixing the cells in the plate.
Note: Cold 2x Freezing medium (60% FBS, 20% TeSR-E8 medium, 20% DMSO
3.8 Place the total 1 mL mixture of cells+medium in a cold cryovial.
3.9 Place the cryovial in a MrFrosty container or in a Styrofoam rack at - 80°C overnight and transfer to liquid nitrogen the next day.

Feeder-dependent culture conditions

1. Preparation of CF-1 MEF feeder plates

1.1. Coat each well of a 6-well plate with 1mL of sterile 0.2% 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 37°C.
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 MEF-cell 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 ES/ iPS cell culture medium

2.1. To prepare 500 mL of hESC medium:

- 100 mL KnockOut Serum Replacement
- 5 mL 100x Glutamine
- 5 mL 100x Non-Essential Amino Acids
- 500 µL 10 µg/mL β-FGF
- 500 µL 55 mM beta-Mercaptoethanol
- 1000 µL Primocin or 5 mL 100x Pen/Strep
- Make up volume to 500 mL with DMEM/F12

2.2 Mix all the reagents in the top cup of a 500 mL filter system (0.22µm) and filter.

3. Thawing/plating cryopreserved human iPS cells

3.1. Prepare 2.5 mL of hESC medium + 10 µM Rock Inhibitor.
3.2. Aspirate the medium from a well of a 6-well feeder plate, wash once with 1x PBS, and add 1 mL of hESC medium + Rock Inhibitor. Label the plate with the name of the clone, passage number and date, and place it in a CO_2 incubator.
3.3. Bring the cryovial on dry ice to the tissue culture room.
3.4. Quickly thaw the hiPSCs in a 37°C waterbath by gently shaking the cryovial continuously until only a small frozen pellet remains.
3.5. Wipe the cryovial with a Kimwipe sprayed with 70% ethanol and place it into a biosafety cabinet.
3.6. Transfer the cells to a 50 mL conical tube.
3.7. Add 5 mL of cold hESC medium dropwise while swirling the conical tube with the cells.
3.8. Centrifuge the cells for 5 min at 300g at 4°C.
3.9. Aspirate off the medium and add 1mL of hESC medium + Rock Inhibitor.
3.10. Gently flick the conical tube to resuspend the cells and transfer them to the well of the feeder plate using a 5 mL serological pipette.
Note: Feeder plates should be prepared not more than a week prior to thawing cells
3.11. Place the plate in the incubator and move the plate back and forth and side to side twice to spread the clumps evenly in the well.
3.12. Change medium daily. Usually, after 1-2 weeks the cells are ready to be split.

4. Passaging human iPS cells

4.1. Aspirate the medium from the hiPSC culture.
4.2. Wash once with 2 mL of 1x PBS.
4.3. Add 1 mL of 0.5 mM EDTA (in PBS) per well of a 6-well plate and incubate the cells for 3-5 min at RT in a safety cabinet.
4.4. Observe the cells under the microscope. The cells at the edge of the colonies will start to separate and round up.

4.5. Aspirate the EDTA and add 1 mL of hESC medium.
4.6. Scrape the cells from the bottom of the well until the colonies are all floating and pipette up and down 2-3 times (with the 1000 µL pipette set to 800 µL) to break the colonies into small clumps.
Note: Cells are passaged as clumps of small to medium size containing about 50-200 cells.
4.7. Transfer the desired dilution to the wells of the new feeder plate (usually at 1:3 – 1:10 ratio).
Note; Feeder plates should be prepared not more than a week prior to passaging
4.8. Place the plate in the incubator and move the plate back and forth and side to side twice to spread the clumps evenly in the well.
Note: Once the colonies are at an optimal density for passaging, (the cells can be split. On average, iPSCs can be passaged once every week.

5. Cryopreserving human iPS cells

5.1. Label cryovials as needed, based on 1 vial per well of a 6-well plate, and pre-chill them in a – 20°C freezer.
5.2. Aspirate the medium from the hiPSC culture.
5.3. Wash once with 2 mL of 1x PBS.
5.4. Add 1 mL of 0.5 mM EDTA (in PBS) per well of a 6-well plate and incubate the cells for 3-5 min at RT in biosafety cabinet.
5.5. Observe the cells under the microscope. When the cells at the edge of the colonies start to separate and round up, aspirate the EDTA and add 0.5 mL of cold hESC medium.
5.6. Scrape the cells from the bottom of the well until the colonies are all floating.
Note: Do not pipette up and down when freezing hiPSCs!
5.7. Add 0.5 mL of cold 2x freezing medium dropwise while mixing the cells in the plate.
Note: Cold 2x Freezing medium (60% FBS, 20% hESC medium, 20% DMSO)
5.8. Place the total 1 mL in a cold cryovial.
5.9. Place the cryovial in a MrFrosty container or in a Styrofoam rack at -80°C overnight and transfer liquid nitrogen the next day.

References

1. Gonzalez, F., Boue, S., Belmonte, J. C. I., Methods for making induced pluripotent stem cells: reprogramming à 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. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. (2007) *Cell*. 131(5):861-72