

Human Induced Pluripotent Stem Cells (from Normal, Female, Human Fibroblasts)

Applied StemCell, Inc. provides Control Human Induced Pluripotent Stem (iPS) cells at low passages. These pluripotent cells were generated from normal human skin fibroblasts using episomal reprogramming methods. This method allows the transient expression of human transcription factors (OCT4, SOX2, KLF4, and c-MYC) that initiate the reprogramming process. 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, OCT4, SOX2, SSEA4, TRA-1-60, and TRA-1-81 (Figure 1) and normal female karyotype (Figure 2). The ASE-9209 control human iPSC line can be used for CRISPR/Cas9 genome editing and differentiation to somatic lineages *in vitro*. Detailed protocols for thawing, culturing under feeder-free conditions, and cryopreservation of these iPS cells are provided.

Reprogramming Method: Episomal

Passage: P20

Tissue: Dermal skin (fibroblasts)

Age: 47 years

Sex: Female

Race: Caucasian

Clinical information: Normal

Shipping conditions: Dry ice

Catalog #	Product	Size
ASE-9209	Dermal skin (fibroblasts)	1 x 10 ⁶ cells/vial Additional package sizes are available upon request

Intended Use

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

Quality Assurance

Each lot of human iPS cells has been tested for growth and viability following recovery from cryopreservation, morphology, immunohistochemistry for pluripotency markers (OCT4, SOX2, SSEA4, TRA-1-60, TRA-1-81); and for the absence of bacteria, fungi, mycoplasma (CoA available upon request).

Storage Instructions and Stability

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

Precautions

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.

Directions for Use

Required Materials (Not Provided)

Catalog #	Product	Size
100-0276	mTeSR™ Plus + Supplement	500 ml
354277	Matrigel®, Corning	5 ml
04-0012-02	Rock Inhibitor Y-27632	5 mg

Protocol for Thawing human iPSCs

1. Prepare Matrigel® or Geltrex coated 6-well plates in advance, following vendor's instructions.
Note: Prepare Matrigel or Geltrex plates the day before and not more than 3 days prior to thawing. Before transferring the cells, the Matrigel must be aspirated.
2. Prepare 6 mL of mTeSR-plus medium (mix of 4.8 mL basal medium and 1.2 mL 5x supplement) + Primocin (1:500 dilution) + 10 µM Rock Inhibitor.
Note: Besides Rock inhibitor, the clone R can be used.
3. Add 1 mL of mTeSR-plus medium + Rock Inhibitor + Primocin in one well of a Matrigel®-coated plate. Prepare 1 well for each vial of frozen cells.
4. Bring the cryovial on dry ice to the tissue culture room.
5. Quickly thaw the iPSCs in a 37°C water bath, by gently shaking the cryovial continuously until only a small piece of ice remains.
6. Wipe the cryovial with a paper towel sprayed with 70% ethanol and place it into a biosafety cabinet.
7. Add 9 mL of mTeSR-plus medium + Primocin to a 15 mL conical tube.
8. Using a 1 mL pipette transfer the cells to the 15 mL conical tube dropwise while swirling the conical tube.
9. Centrifuge the cells for 3 minutes at 200 RCF and at room temperature.
10. Aspirate off the medium, and add 3 mL mTeSR-plus medium + Rock Inhibitor + Primocin.
11. Gently flick the conical tube to resuspend the cells and transfer them to the 1 well of the Matrigel® or Geltrex plate using a 5 mL serological pipette.
12. 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 wells.
13. Medium can be changed 2 days after thawing. After the 2 days, if the number of attached colonies is still low (less than 5% confluency), change half of the medium (aspirate 1 mL and add 1 mL of mTeSR-plus medium + Primocin).
14. Once colonies are stabilized, change the medium daily. Usually, within 1 week the cells are ready to be split.
15. When the ASE-9209 hiPSC colonies are big or close enough to merge, the cells need splitting/passaging.

Protocol for Passaging and Seeding human iPSCs

1. Aspirate the medium from the hiPSC culture.
2. Wash once with 1 mL of 0.5 mM EDTA (in PBS).
3. Aspirate the EDTA and add 1 mL of 0.5 mM EDTA (in PBS) per well of a 6-well plate and incubate the cells for 3 min in a 37°C incubator.
4. Observe the cells under a microscope until the cells at the edge of the colonies start to separate and round up.
5. With the cells still attached, aspirate the EDTA and add 1 mL of mTeSR-plus medium.
6. 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.

7. Transfer the desired dilution to the wells of the new Matrigel®-coated plate (usually around a 1:3 splitting ratio).

Note: Prepare Matrigel plates (or any other basement matrix) not more than a week prior to passaging cells.

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 all the wells.

Note: Human iPSCs are passaged as clumps of 50-200 cells, rather than single cells. Cells need to be passaged before the colonies are large enough to merge with one another.

Protocol for Freezing human iPSCs

1. Label the cryovial as needed, based on 1 vial per well of a 6-well plate, and pre-chill them in a 4°C freezer.
2. Aspirate the medium from the hiPSC culture.
3. Wash once with 1 mL of PBS.
4. Aspirate the PBS and add 1 mL of 0.5 mM EDTA (in PBS) per well of a 6-well plate and incubate the cells for 3-5 minutes in a 37°C incubator.
5. Observe the cells under a microscope. After 1-2 minutes the cells at the edge of the colonies will start to separate and round up.
6. Once round colonies are observed, aspirate the EDTA (even if it hasn't been 5 minutes of incubation).
7. Add 1 mL of cold freezing medium, CS10 and scrape the cells from the bottom of the well until the colonies are floating.

Note: The freezing medium must remain at 4°C until usage.

8. Pipette the cells once or twice to break up any big clumps before transferring the suspension to a cryovial.
9. Transfer the cell suspension into the pre-chilled and labeled cryovial.
10. Place the cryovial in a CoolCell® Freezing Container or in a Styrofoam rack at -80°C overnight, and transfer to liquid nitrogen the next day.

Characterization of iPSC line ASE-9209

Pluripotency Marker Analysis

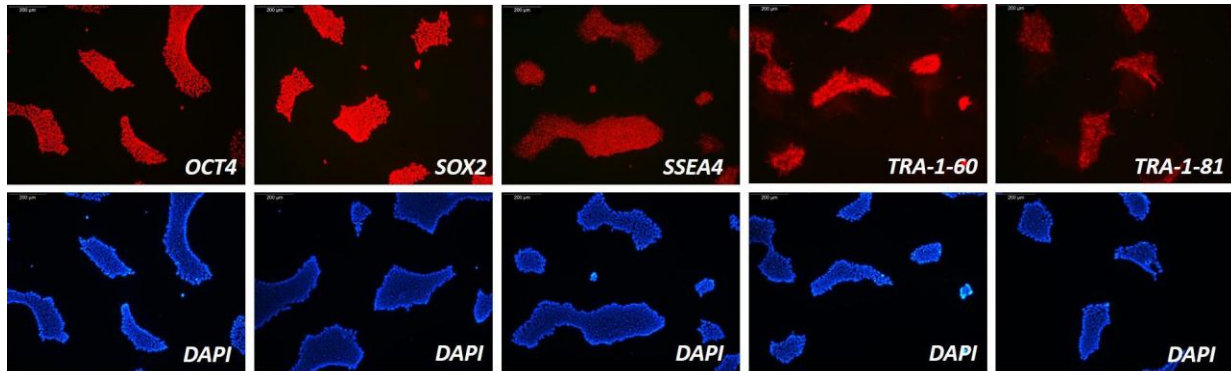


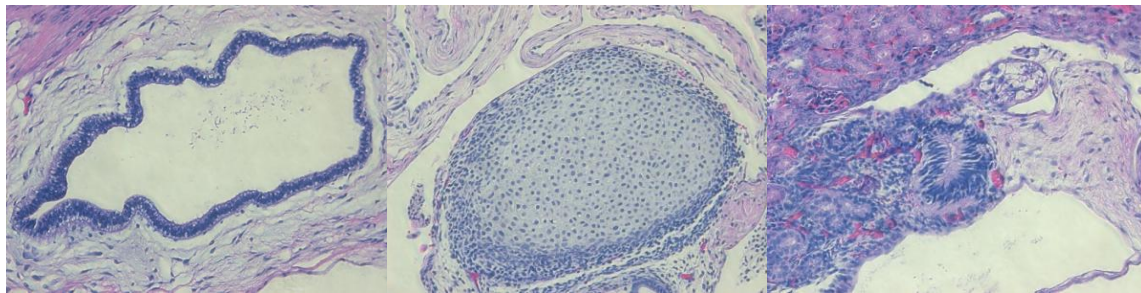
Figure 1a. Expression of pluripotency markers. ASC 9209 iPS cell line expresses common iPSC biomarkers (OCT-4, TRA-1-60, TRA-1-81 SSEA-4 and SOX-2). The corresponding DAPI staining is below each image. All images were taken at 10x magnification.

Karyotype Analysis



Figure 2. Karyotype analysis to rule out genetic aberrations. Cytogenic analysis was performed on G-banded metaphase cells from human iPSC line, ASE-9209 at passage 15. This iPSC line demonstrates a normal female karyotype.

Teratoma Formation



EN: Gland (200x)

ME: Cartilage (200x)

EC: Neuronal Rosette (200x)

Figure 3. Histological analyses using H&E staining of kidney and testis teratomas from mice injected with the ASE-9209 iPSC line. Abbreviations: EN: endoderm; ME: mesoderm; EC: ectoderm.

Differentiation to Motor Neurons

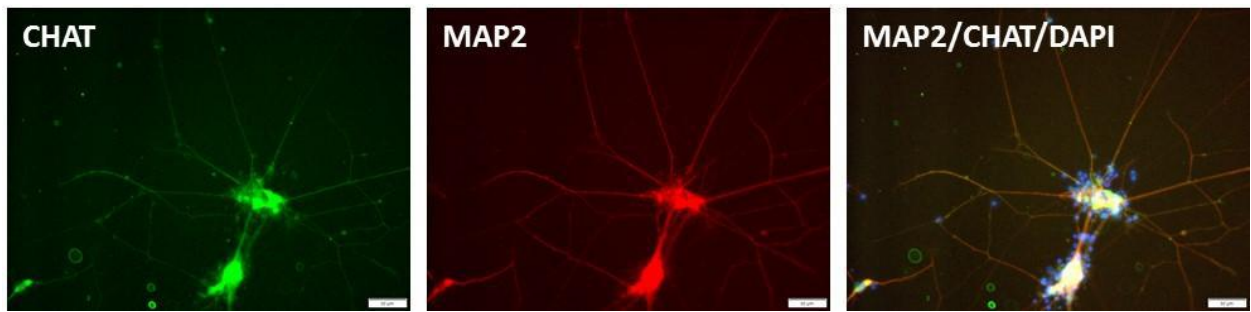


Figure 4. Immunocytochemical staining for Neuronal Differentiation. Motor neuron marker, ChAT (green) and neuronal marker, MAP2 (red) motor neurons differentiated from control iPSC line, ASE-9209 at 7 days post-thaw. DAPI (blue) was used for nucleus staining.

Related Products

Catalog #	Product	Size
ASE-9215	Human iPSCs from PBMCs (Normal, Male, Caucasian, Adult)	1 X 10 ⁶ cells/vial
ASE-9216	Human iPSCs from Dermal Fibroblasts (Normal, Male, Caucasian, Neonate)	1 X 10 ⁶ cells/vial
ASE-9211	Human iPSCs from Fibroblasts (Normal, Male, African American, Neonate, NIST line)	1 X 10 ⁶ cells/vial

Technical Support

CONTACT US

For more information or assistance, contact Customer Service at:

- Email: info@appliedstemcell.com
- Direct line: (408) 773-8007

WEBSITE RESOURCES

Visit appliedstemcell.com for technical resources and information including:

- Safety Data Sheets (SDS)
- Certificates of Analysis (when available)
- FAQs
- Product literature
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