Applied StemCell, Inc.
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Datasheet

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

Product Information

Catalog Number	**ASE-9209**
Description	Applied StemCell, Inc. provides Control Human Induced Pluripotent Stem (iPS) cells at low passages (p16). 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 #	P16
Tissue	Dermal skin (fibroblasts)
Age	47 years
Sex	Female
Race	Caucasian
Clinical information	Normal
Quantity	0.5 x 10^6 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, morphology, immunohistochemistry for pluripotency markers (*OCT4, SOX2, SSEA4, TRA-1-60, TRA-1-81*, and AP staining); and for the absence of bacteria, fungi, mycoplasma (CoA available upon request).

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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-9209

Pluripotency Marker Analysis

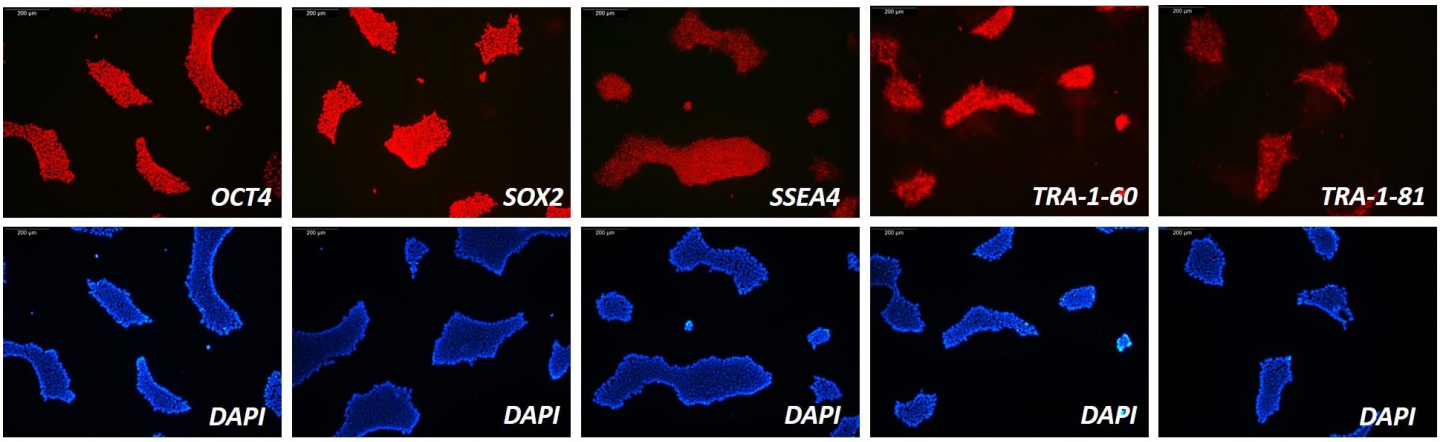


Figure 1a. Expression of pluripotency markers. ASC 9209 iPS cell line expresses common iPSC biomarkers (*OCT-4, TRA-1-60, TRA-1-81 SSEA-4 and SOX-2*). The corresponding DAPI staining is below each image. All images were taken at 10x magnification.

Karyotype Analysis

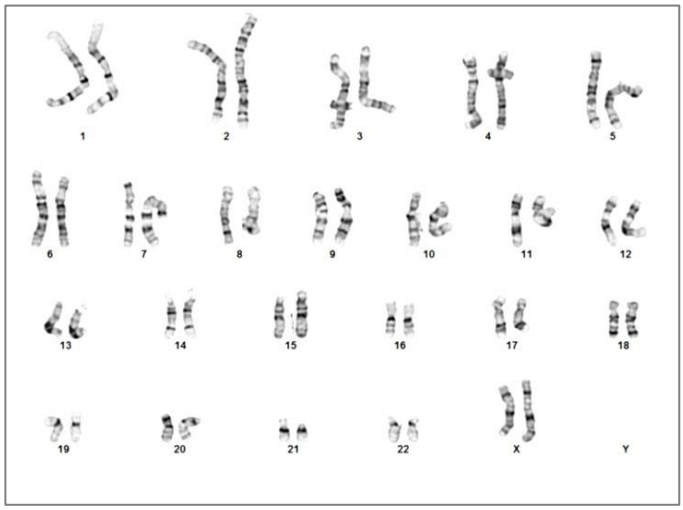
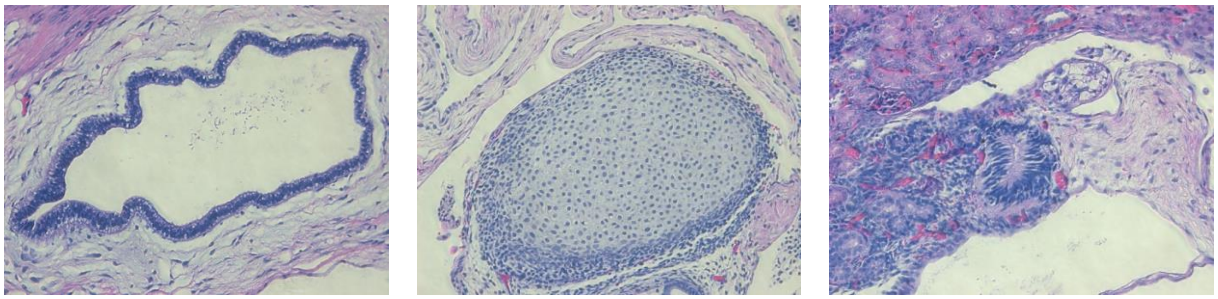


Figure 2. Karyotype analysis to rule out genetic aberrations. Cytogenic analysis was performed on G-banded metaphase cells from human iPSC line, ASE-9209 at passage 15. This iPSC line demonstrates a normal female karyotype.

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

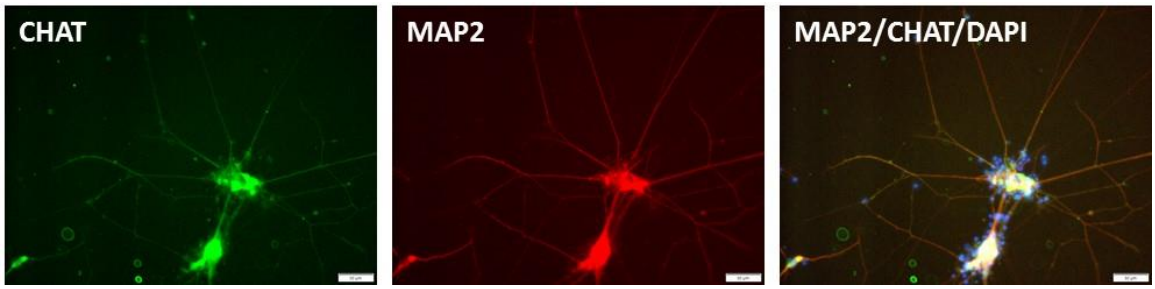


Figure 4. Immunocytochemical staining for motor neuron marker, ChAT (green) and neuronal marker, MAP2 (blue) motor neurons differentiated from control iPSC line, ASE-9209 at 7 days post-thaw. DAPI (blue) was used for nucleus staining.

CRISPR Genome Editing in ASE-9209

Figure 5. Next generation sequencing of pooled clones from control iPSC ASE-9209 transfected with gRNA targeting a gene-of-interest (highlighted in orange) and Cas9 shows indel formation, indicating gRNA-mediated cleavage in this region. WT (Ref Seq): wild type reference sequence.

Media and Material Required but not Provided

iPSC Media and Materials for Feeder-free Culture System

- StemFlex™ Medium, ThermoFisher Scientific, Cat# A3349401
- Matrigel®, Corning, Cat# 354277
- Rock Inhibitor Y-27632, Stemgent, Cat# 04-0012-02
- KnockOut™ Serum Replacement, ThermoFisher Scientific, Cat# 10828028
- Dimethyl sulfoxide (DMSO), Sigma, Cat# D2650)
- 0.5M EDTA in PBS, ThermoFisher Scientific, Cat# 15575-020
- Cell Scraper, Corning, Cat# 3010

Protocol

Feeder-free culture conditions

1. Thawing human iPSCs using a feeder-free protocol

1.1 Prepare Matrigel® coated 6-well plates in advance, following vendor's instructions.
1.2 Prepare 6 mL of StemFlex™ medium + 10 µM Rock Inhibitor.
1.3 Add 1 mL of StemFlex™ medium + Rock Inhibitor in one well of a Matrigel®-coated plate. Prepare 3 wells for each vial of frozen cells.
1.4 Bring the cryovial on dry ice to the tissue culture room.
1.5 Quickly thaw the iPSCs in a 37°C water bath, by gently shaking the cryovial continuously until only a small piece of ice remains.
1.6 Wipe the cryovial with a paper towel sprayed with 70% ethanol and place it into a biosafety cabinet.
1.7 Add 9 mL of StemFlex™ medium to a 50 mL conical tube.
1.8 Using a 5 mL serological pipette transfer the cells to the 50 mL conical tube dropwise while swirling the conical tube.
1.9 Centrifuge the cells for 5 min at 200g at 4°C.
1.10 Aspirate off the medium, and add 3 mL StemFlex™ medium + Rock Inhibitor.
1.11 Gently flick the conical tube to resuspend the cells and transfer them to the 3 wells of the Matrigel® plate using a 5 mL serological pipette.
Note: Prepare Matrigel plates the previous day and not more than 3 days prior to thawing.

1.12 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.
1.13 Change medium every day. For medium change, aspirate off the spent medium and add 2 mL of fresh StemFlex™ medium in each well.
1.14 When the ASE-9209 hiPSC colonies are big or close enough to merfe, the cells need splitting/ passaging.
Note: The hiPSCs grow faster in StemFlex™ than in mTeSR1. Usually the cells need passaging every 3-7 days. Long term culture without passaging compromises the cell quality.

2. Passaging/ splitting human iPSCs using EDTA

2.1 Aspirate the medium from the hiPSC culture.
2.2 Wash once with 1 mL of 0.5 mM EDTA (in PBS).
2.3 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 2-3 min in a 37°C incubator.
2.4 Observe the cells under a microscope until the cells at the edge of the colonies start to separate and round up.
2.5 With the cells still attached, aspirate the EDTA and add 1 mL of StemFlex™ medium.
2.6 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.
2.7 Transfer the desired dilution to the wells of the new Matrigel™-coated plate (usually around a 1:6 splitting ratio).
Note: Prepare Matrigel plates (or any other basement matrix) not more than a week prior to passaging cells
2.8 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: hiPSCs 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.

3. Cryopreserving human iPS cells

3.1 Prepare fresh 2x freezing medium according to the recipe below, and keep it on ice.
- 80% Knockout™ Serum Replacement
- 20% DMSO

3.2 Label the cryovials as needed, based on 2 vials per well of a 6-well plate, and pre-chill them in a – 20°C freezer.
3.3 Aspirate the medium from the hiPSC culture.
3.4 Wash once with 1 mL of 0.5 mM EDTA (in PBS).
3.5 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 2-3 min in a 37°C incubator.

3.6 Observe the cells under a microscope until the cells at the edge of the colonies start to separate and round up.
3.7 With the cells still attached, aspirate the EDTA and add 1 mL of StemFlex™ medium.
3.8 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.
3.9 Add 1 mL of cold 2x freezing medium dropwise while mixing the cells in the plate.
3.10 Aliquot the cell suspension in 2 pre-chilled and labeled cryovials: 1 ml in each vial.
3.11 Place the cryovial in a MrFrosty container or in a Styrofoam rack at - 80°C overnight, and transfer to liquid nitrogen the next day.