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

Datasheet

Human Induced Pluripotent Stem Cells
(from Normal, Male, Human Dermal Fibroblasts (HDF))

Product Information

Catalog Number	**ASE-9216**
Description	Applied StemCell, Inc. provides Control Human Induced Pluripotent Stem (iPS) cells at low passages (p8). These pluripotent cells were generated from normal human dermal fibroblasts (HDF) using a Sendai virus reprogramming method. This method allows the transient expression of human transcription factors *(OCT4, SOX-2, 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 normal male karyotype (Figure 1 and 2) and pluripotency (Figure 3 and 4). The ASE-9216 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	Sendai Virus
Passage #	p8
Tissue	Foreskin, Human Dermal Fibroblasts (HDF)
Age	Neonatal
Sex	Male
Race	Caucasian
Clinical information	Normal
Quantity	1 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	The human iPS cells have been tested for normal male karyotype and pluripotency (expression of Oct4, Sox2, Tra-1-81 and teratoma formation).
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.

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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-9216

Karyotype Analysis

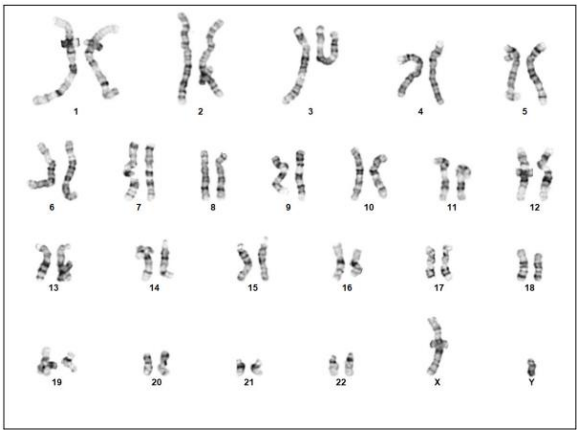

Figure 1. Karyotype analysis of p6 iPSC line ASE-9216 to rule out genetic aberrations. This iPSC line demonstrates a normal male karyotype. Cytogenetic analysis was performed on twenty G-banded metaphase cells from human iPSC line, ASE-9216 at passage 6. Eighteen cells demonstrated an apparently normal male karyotype, and two cells demonstrated non-clonal chromosome aberrations, which are most likely artifacts of culture.

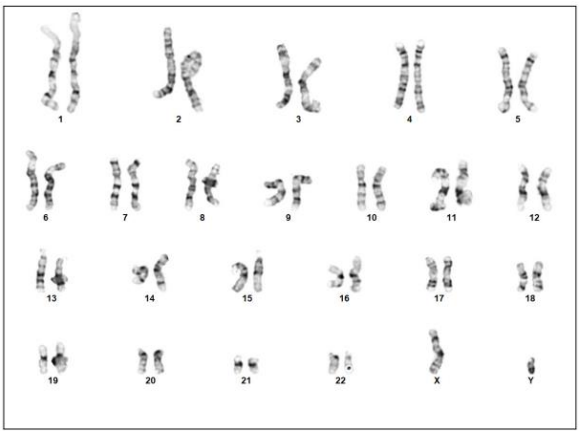

Figure 2. Karyotype analysis of p15 iPSC line ASE-9216 to rule out genetic aberrations. This iPSC line demonstrates a normal male karyotype. Cytogenic analysis was performed on twenty G-banded metaphase cells from human iPSC line, ASE-9216 at passage 15. All twenty cells demonstrated an apparently normal male karyotype.

Pluripotency Marker Analysis

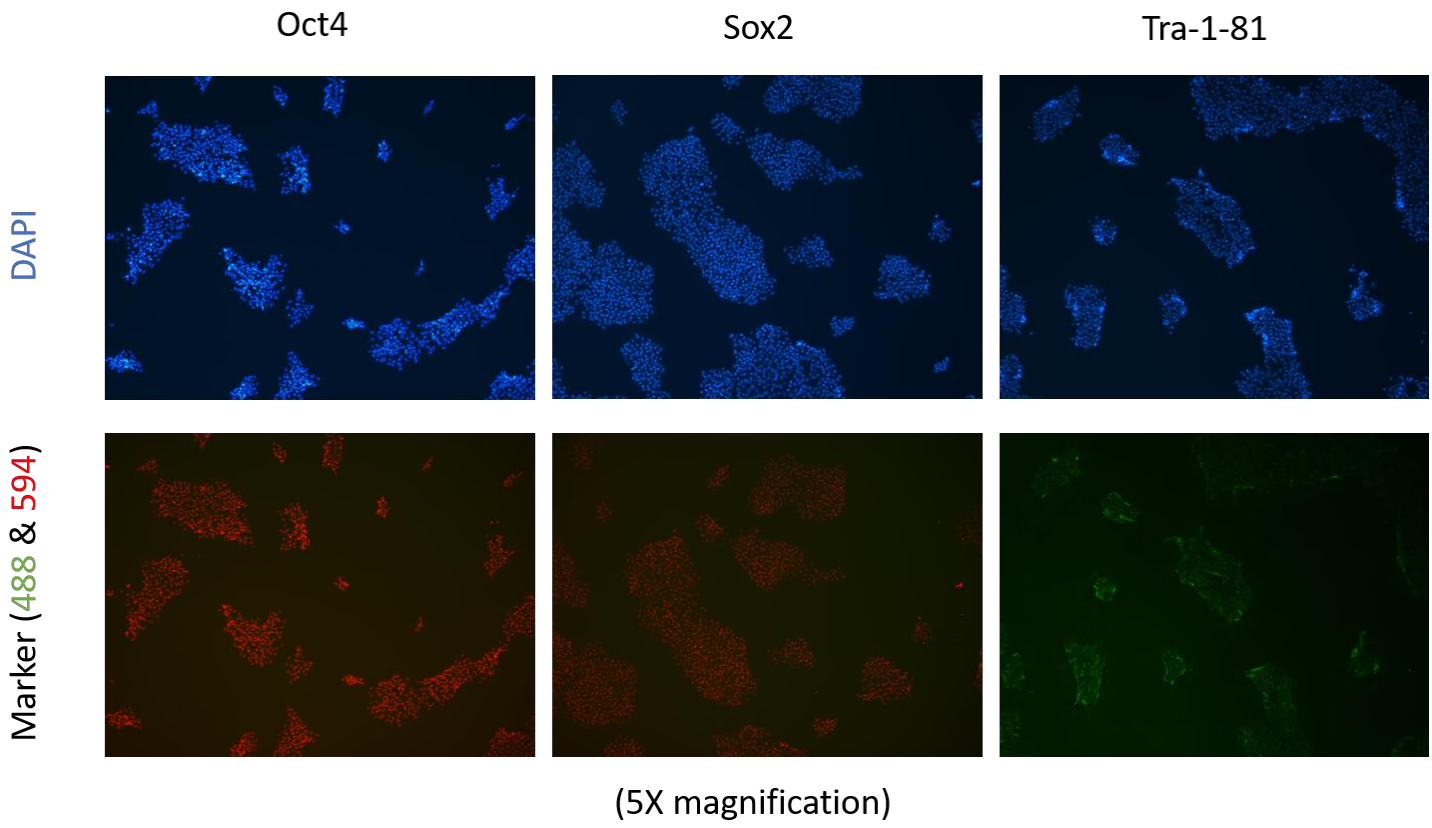

Figure 3: Expression of pluripotency markers. The ASE-9216 iPS cell line (P9) expresses common iPSC biomarkers (Oct4, Sox2, Tra-1-81). The corresponding DAPI staining is above each marker image. All images were taken at 5X magnification.

Teratoma Formation

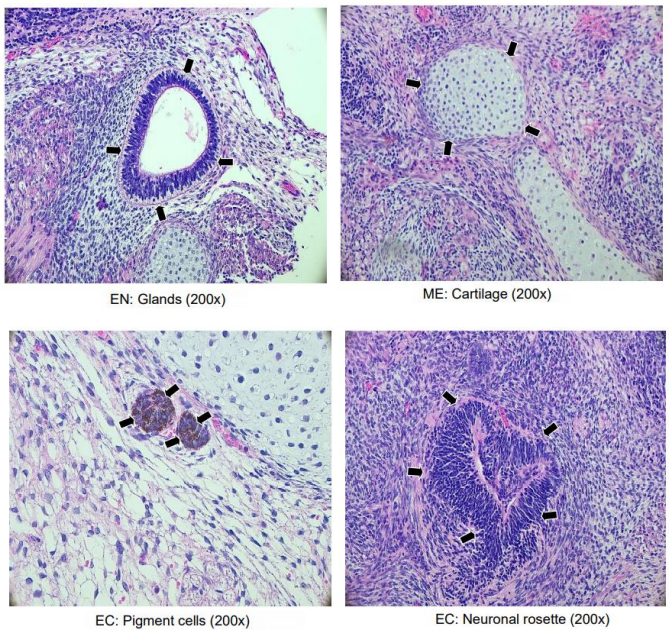

Figure 4: Histological analysis of a teratoma in the testis that was injected with the ASE-9216 cells. Variously differentiated tissues that represent the three germ layers are shown and indicated by arrow heads. Abbreviations: EN, endoderm; ME, mesoderm; EC, ectoderm. (Forty-seven days post inoculation) Histology results indicated that there was a teratoma composed of scattered regions of differentiated cells and a large population of undifferentiated neoplastic cells. These differentiated cells represent all three germ layers, suggesting that these injected cells are pluripotent.

Media and Material Required but not Provided

- mTeSR-Plus Medium, StemCell Technologies, Cat# 05825
- Matrigel®, Corning, Cat# 354277
- ROCK Inhibitor (Y-27632), Sigma-Aldrich, Cat# SCM075
- CryoStor® CS10, StemCell Technologies, Cat# 7930
- 0.5M EDTA in PBS, ThermoFisher Scientific, Cat# 15575-020
- PBS, Life Technologies, Cat# 14190136
- 100 µg/mL Primocin™, Invivogen, Cat# ant-pm-2 or 1% Penicillin- Streptomycin, Life Technologies, Cat# 15140
- Cell Scraper, VWR International, Cat# 75799-938
- Corning® CoolCell® FTS30, Corning®, Cat# 432006

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 mTeSR-plus medium + 10 µM Rock Inhibitor.
1.3 Add 1 mL of mTeSR-plus medium + Rock Inhibitor in one well of a Matrigel®-coated plate. Prepare 2 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 mTeSR-plus medium to a 15 mL conical tube.
1.8 Using a 1 mL pipette transfer the cells to the 15 mL conical tube dropwise while swirling the conical tube.
1.9 Centrifuge the cells 3 min at 200 RCF and at room temperature.
1.10 Aspirate off the medium, and add 4 mL mTeSR-plus medium + Rock Inhibitor.
1.11 Gently flick the conical tube to resuspend the cells and transfer them to the 2 wells of the Matrigel® plate using a 5 mL serological pipette.
Note: Prepare Matrigel plates the day before and not more than 3 days prior to thawing. Before transferring the cells, the Matrigel must be aspirated.

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 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 fresh medium).
1.14 Once colonies are stabilized, change the medium daily. Usually, within 1 week the cells are ready to be split.
1.15 When the ASE-9216 hiPSC colonies are big or close enough to merge, the cells need splitting/ passaging.

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 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 mTeSR-plus 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 1:3 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 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.
3.2 Aspirate the medium from the hiPSC culture.
3.3 Wash once with 1 mL of PBS.
3.4 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.
3.5 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.
3.6 Once round colonies are observed, aspirate the EDTA (even if it hasn't been 5 minutes of incubation).
3.7 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.

3.8 Pipette the cells once or twice to break up any big clumps before transferring the suspension to a cryovial.
3.9 Transfer the cell suspension into the pre-chilled and labeled cryovial.
3.10 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.