

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

Control Human Induced Pluripotent Stem cells (iPSCs) at low passage (p8) provided by Applied StemCell Inc. This iPSC line was generated from normal Human Dermal Fibroblasts (HDFs) using a Sendai virus method. This method allows the expression of human transcription factors (Oct-4, Sox-2, KLF4, and c-MYC) that initiate the reprogramming process. The resulting induced pluripotent cells were selected using morphological criteria without the use of either fluorescent markers or drug selection.

Catalog #	Product	Size
ASE-9216	Human iPSCs (from Normal, Male, HDFs)	1.0 × 10 ⁶ cells/vial

Parental Tissue Information	
Reprogramming Method	Sendai Virus
Passage Number	p8
Tissue	Human Dermal Fibroblasts (HDFs)
Sex	Male
Age	Neonatal
Race	Caucasian
Clinical Information	Normal

Intended Use

The ASE-9216 control human induced pluripotent cell line can be used for CRISPR-CAS9 genome editing and differentiation into somatic lineages *In vitro*.

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

Quality Assurance

Each lot of ASE-9126 human iPSCs has been tested for normal male karyotype and pluripotency (expression of Oct-4, Sox-2, Tra-1-81 and teratoma formation)

All quality control test results are reported on a lot-specific Certificate of Analysis, available at appliedstemcell.com or upon request.

Storage Instructions and Stability

Store in liquid nitrogen freezer immediately upon receipt.

The iPSC line is stable for 6 months starting from the arrival date of the product. Every 6 months cells should be tested for pluripotency and differentiation potential to ensure they are viable for experimental use.

Precautions

The safety and efficacy of this product in diagnostic or other clinical uses have not been established. This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials while they are submerged in a liquid nitrogen freezer. During rapid 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. When retrieving the cell vial from a liquid nitrogen freezer, carefully twist open the cap to release any built up nitrogen gas prior to thawing the cells.

Refer to the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Directions for Use

Required Materials (Not Provided)

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

Protocol for Thawing human iPSCs

1. Prepare Matrigel® or coated 6-well plates in advance, following vendor's instructions.
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.
2. Prepare 6 mL of mTeSR-plus medium + 10 µM Rock Inhibitor.
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.
4. Retrieve the cryovial from the liquid nitrogen freezer and place the cryovial on dry ice to transfer 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 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 min at 200 RCF and at room temperature.
10. Aspirate off the medium, and add 4 mL mTeSR-plus medium + Rock Inhibitor.
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.

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 fresh medium).
14. Once colonies are stabilized, change the medium daily. Usually, within 1 week the cells are ready to be split.
15. When the ASE-9215 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.

Data

Karyotype Analysis

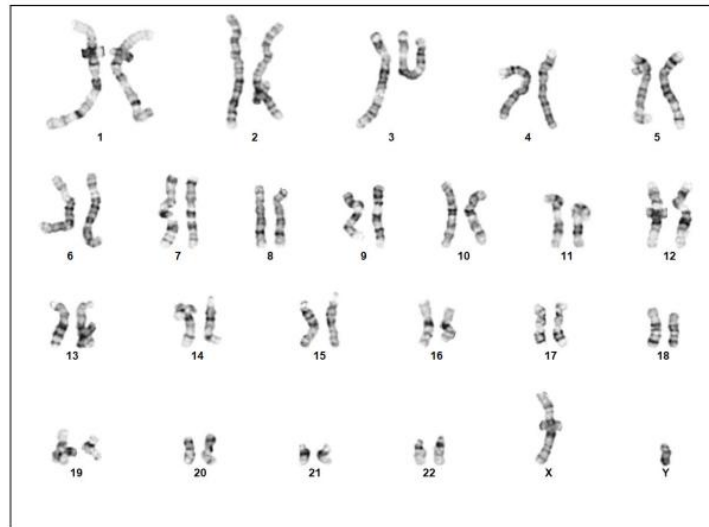


Figure 1: Karyotype analysis of p6 iPSC line ASE-9216 to rule out genetic aberrations. This iPSC line demonstrates a normal male karyotype. Cytogenetic analysis was performed on twenty G-banded metaphase cells from human iPSC line, ASE-9216 at passage 6. Eighteen cells demonstrated an apparently normal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

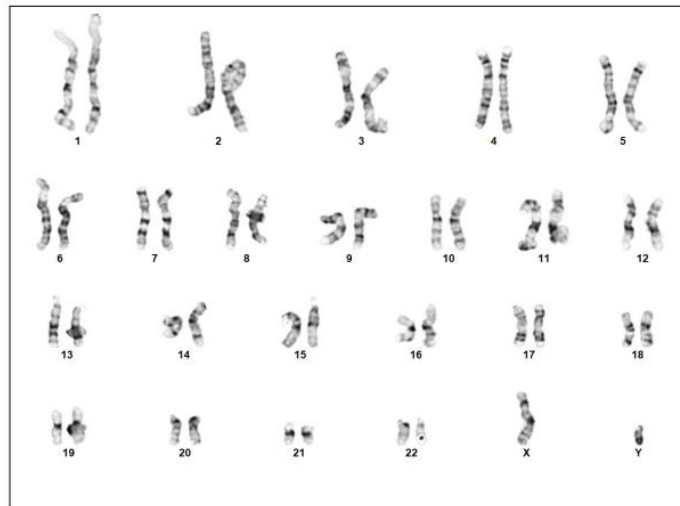


Figure 2: Karyotype analysis of p15 iPSC line ASE-9216 to rule out genetic aberrations. This iPSC line demonstrates a normal male karyotype. Cytogenetic analysis was performed on twenty G-banded metaphase cells from human iPSC line, ASE-9216 at passage 15. All twenty cells demonstrated an apparently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

Pluripotency Marker Analysis

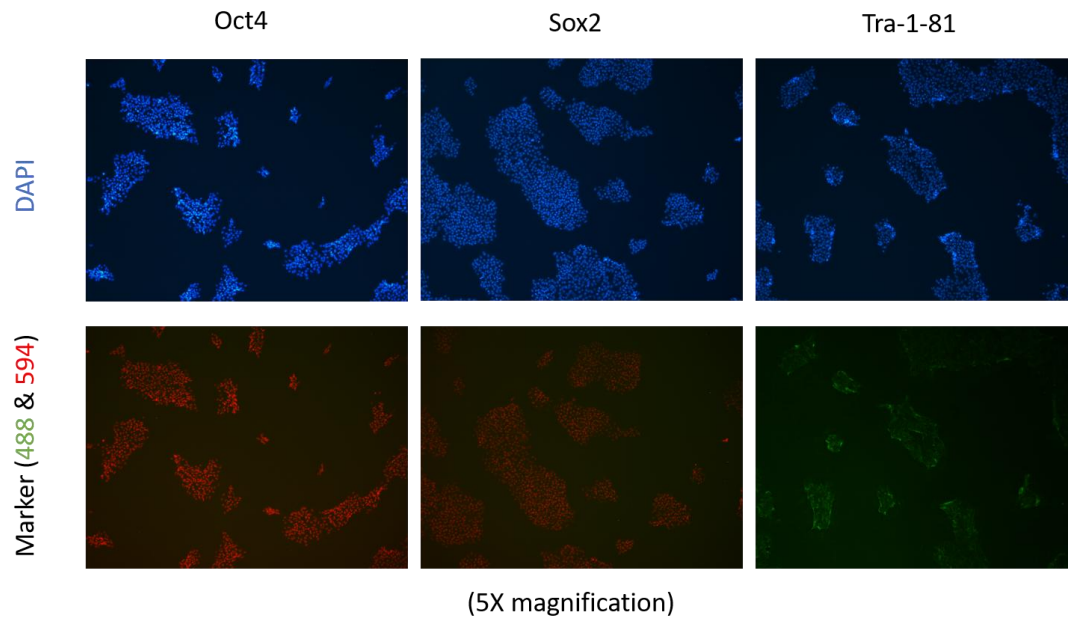


Figure 3: Expression of pluripotency markers. The ASE-9216 iPS cell line (P9) expresses common iPSC biomarkers (Oct4, Sox2, Tra-1-81). The corresponding DAPI staining is above each marker image. All images were taken at 5X magnification.

Teratoma Formation

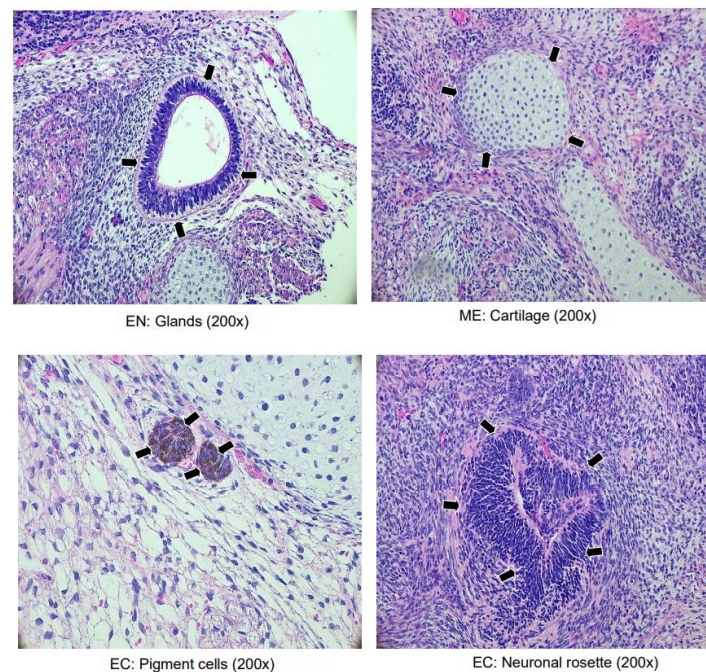


Figure 4: Histological analysis of a teratoma in the testis that was injected with the ASE-9216 cells. Various differentiated tissues that represent the three germ layers are shown and indicated by arrow heads. Abbreviations: EN, endoderm; ME, mesoderm; EC, ectoderm. (Forty-seven days post inoculation) 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.

Related Products

Catalog #	Product	Size
ASE-9215	Human iPSCs from PBMCs (Normal, Male, Caucasian, Adult)	1 X 10 ⁶ cells/vial
ASE-9209	Human iPSCs from Fibroblasts (Normal, Female, Caucasian, Adult)	1 X 10 ⁶ cells/vial
ASE-9217	Human iPSCs (from Normal, Female, PBMCs	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
- Complete contact information

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