Applied StemCell, Inc.
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Datasheet

Human Induced Pluripotent Stem Cells (from Normal, Female, Caucasian PBMCs)

Product Information

Catalog Number	**ASE-9217**
Description	Applied StemCell, Inc. provides Control Human Induced Pluripotent Stem (iPS) cells at low passages (p8). These pluripotent cells were generated from normal, female human peripheral blood mononuclear cells (PBMCs) using a Sendai virus reprogramming method. This method allows the transient expression of human transcription factors 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, including *SSEA4, Oct4, and Sox2* (Figure 2) and normal male karyotype (Figure 3). The ASE-9217 control human iPSC line can be used for CRISPR/Cas9 genome editing and differentiation to somatic lineages *in vitro*. Detailed protocols for thawing, passaging, and cryopreservation of these iPS cells are provided.
Reprogramming Method	Sendai Virus
Passage #	P8
Tissue	PBMC
Age	31
Sex	Female
Race	Caucasian
HLA Types	HLA-A*01:02 HLA-B*08:27 HLA-C*02:07 HLA-DRB1*03:11
Clinical information	Normal
Quantity	1.0 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:

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SSEA4, Oct4, and *Sox2*; karyotyping; and for the absence of bacteria, fungi, mycoplasma (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.

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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-9217

Morphology Analysis

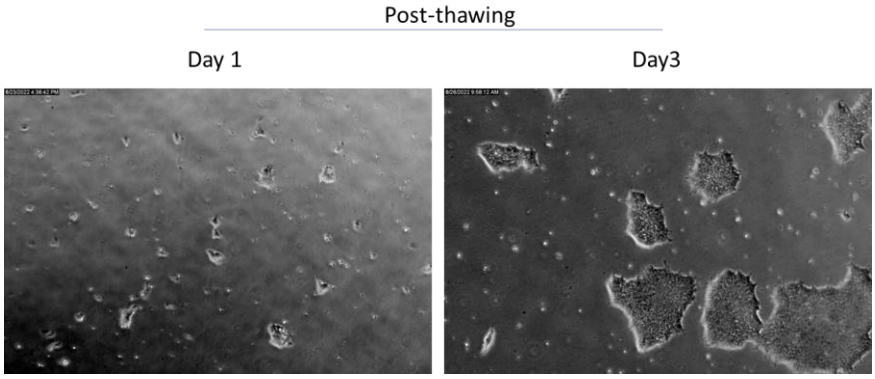

Figure 1. Morphology images of the ASE-9217 iPSC line captured post thaw (Day 1 and 3). The control human iPSCs were generated from normal PBMCs using a Sendai virus method. The image on the left was taken on day 1 and the image on the right was captured day 3.

Pluripotency Marker Analysis

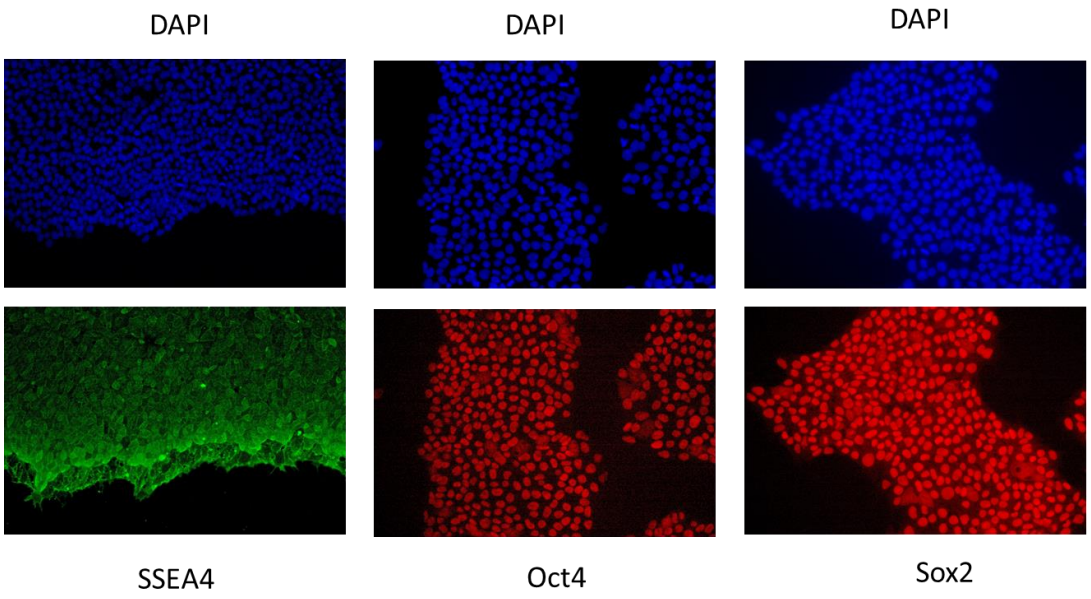

Figure 2. Expression of pluripotency markers. ASE-9217 iPS cell line expresses common iPSC biomarkers (Bottom row: *SSEA4, Oct4, and Sox2*). Top row: Corresponding DAPI nuclear staining.

Karyotype Analysis

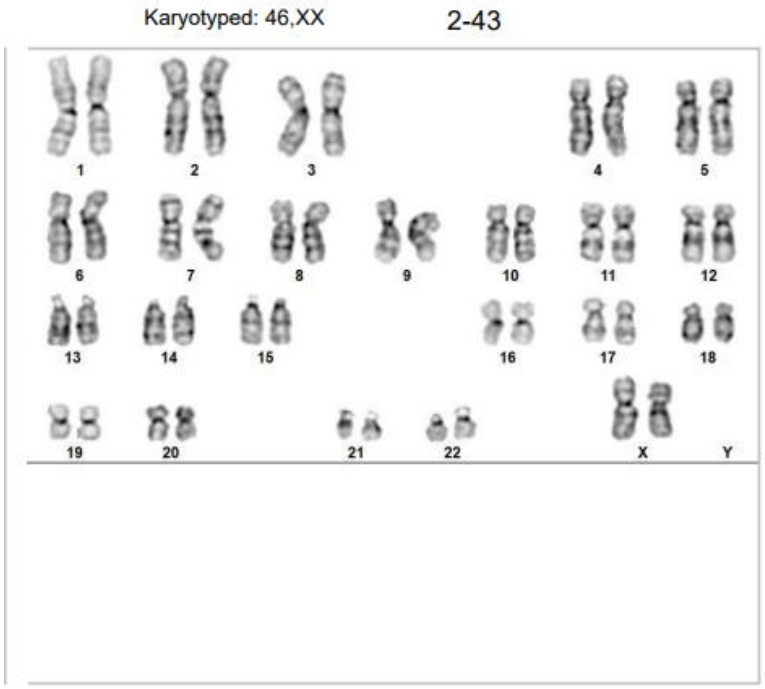

Figure 3. Karyotype analysis to rule out genetic aberrations. The G-banded chromosome analysis of metaphase ASE-9217 cells demonstrate a normal female karyotype.

Directed Differentiation to the Three Germ Layers

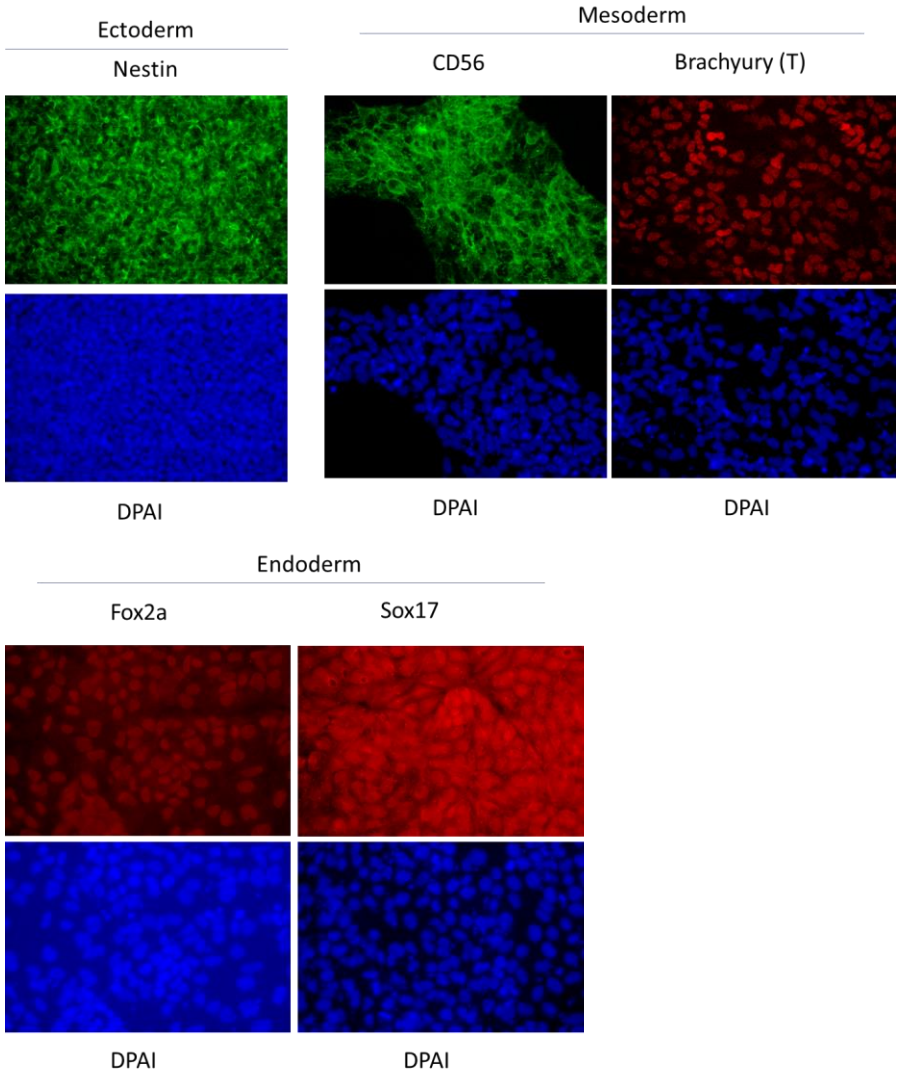

Figure 4. Direct differentiation of ASE-9217 to three germ layers. Immunofluorescent staining for lineage-specific biomarkers of three germ layers after direct differentiation of control hiPSC line, ASE-9217. The ASE-9217 hiPSC line was differentiated to specific lineages of the germ layers using well-established and optimized protocols. Immunostaining for biomarkers of each lineage was performed to confirm lineage commitment. Cells were also co-stained with nuclear marker, DAPI (blue). Ectoderm (EC) marker: Nestin (green); Mesoderm (ME) markers: CD56 (green) and Barchyury (T) (red); Endoderm (EN) markers: Fox2a (red) and Sox17 (red).

Media and Material Required but not Provided

- mTeSR-Plus Medium, StemCell Technologies, Cat# 100-0276
- Primocin, InvivoGen, Cat# ant-pm-05
- Matrigel®, Corning, Cat# 354277
- Geltrex, ThermoFisher, Cat# A1413202
- ROCK Inhibitor (Y-27632), Sigma-Aldrich, Cat# SCM075
- Clone R, StemCell Technologies, Cat# 05889
- CryoStor® CS10, StemCell Technologies, Cat# 7930
- 0.5M EDTA in PBS, ThermoFisher Scientific, Cat# 15575-020
- PBS, Life Technologies, Cat# 14190136
- 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® or Geltrex coated 6-well plates in advance, following vendor's instructions.

1.2 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.

1.3 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.

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 + Primocin 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 for 3 minutes at 200 RCF and at room temperature.

1.10 Aspirate off the medium, and add 3 mL mTeSR-plus medium + Rock Inhibitor + Primocin.

1.11 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.
*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.*

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 mTeSR-plus medium + Primocin).

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-9217 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 + Primocin.
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® or Geltrex-coated plate (usually around a 1:3 splitting ratio).
Note: Prepare Matrigel or Geltrex 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.