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

Datasheet

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

Product Information

Catalog Number	**ASE-9211**
Description	Applied StemCell, Inc. provides Control Human Induced Pluripotent Stem (iPS) cells at low passages (p15). 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, 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 the expression of the pluripotency markers, including *OCT4, SOX2, SSEA4, TRA-1-60, TRA-1-81*, and alkaline phosphatase (AP) activity (Figure 1) and normal male karyotype (Figure 2). The ASE-9211 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 #	P15
Tissue	Dermal skin (fibroblasts)
Age	Neonatal
Sex	Male
Race	African American
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, SOX-2, SSEA-4, TRA-1-60, TRA-1-81*, and AP staining; karyotyping; and for the absence of bacteria, fungi, mycoplasma (CoA available upon request).

Applied StemCell, Inc.
521 Cottonwood Dr. #111, Milpitas, CA 95035
Phone: 866-497-4180 (US Toll Free); 408-773-8007 Fax: 408-773-8238
info@appliedstemcell.com www.appliedstemcell.com

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.

Warranty and Liability

Applied StemCell (ASC) does not extend any expressed or implied warranties. ASC does not provide any warranties of merchantability or suitability for specific product use. Upon purchase, ASC does not provide any warranty that the use of the product will not infringe any patent, copyright, trademark, or other proprietary rights.
The customer acknowledges that the product may be part of a patent application or covered by patent rights in one or more countries. No licenses, expressed or implied, are provided when product is purchased. No license or rights to use a third-party patent, technology, or intellectual property will be included in the purchase of the ASC product. The customer is responsible for obtaining the necessary permissions based on their intend use of the product from third parties that may have a proprietary interest in the product.

Liability Statement for Customers Receiving the ASC Product: Customer agrees that they will be held responsible for any damages, claims, costs, and expenses resulting from any injury (including death), damage, or loss that may be caused by the use or distribution of the ASC product purchased or any derivatives to the extent permitted by law.

Indemnification: Customer agrees to hold harmless Applied StemCell from any damages, claims, costs, or expenses that may result from use of the product and/or derivatives, including injury (including death), damage, or loss.

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-9211

Pluripotency Marker Analysis

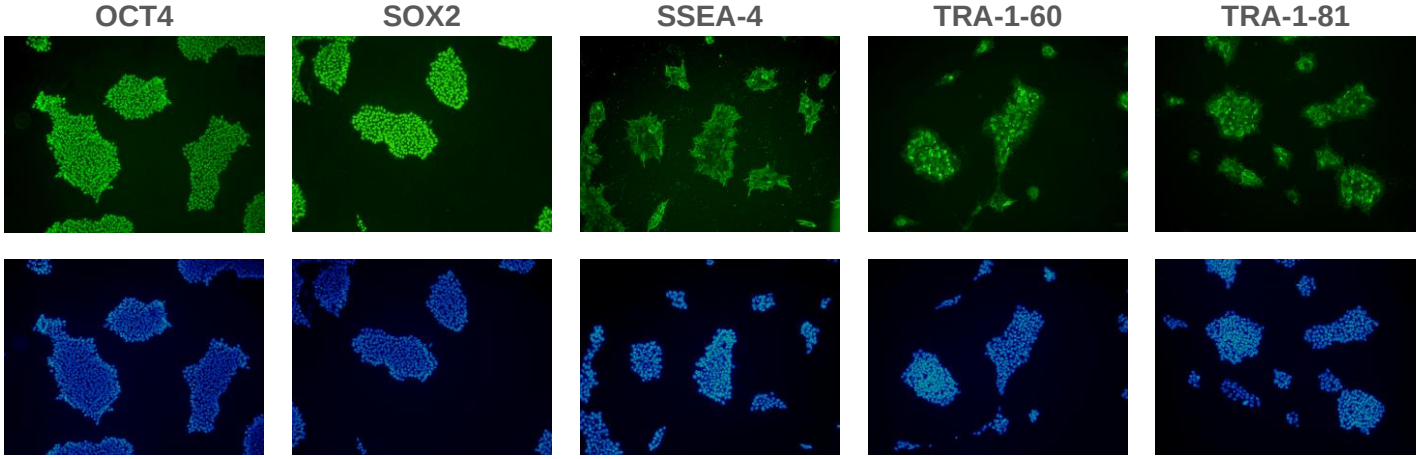


Figure 1a. Expression of pluripotency markers. ASC 9211 iPS cell line expresses common iPSC biomarkers (top row: *OCT-4, SOX2, SSEA-4, TRA-1-60, and TRA-1-81*). Bottom row: Corresponding DAPI nuclear staining. All images were taken at 10x magnification.

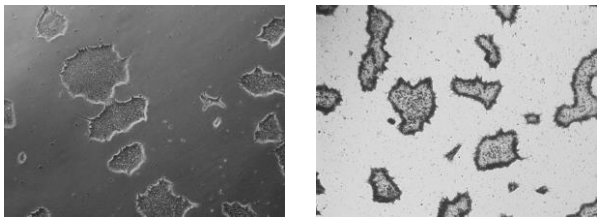


Figure 1b. Alkaline Phosphatase (AP) staining. ASC-9211 iPSCs stain positive for Alkaline Phosphatase: a typical unstained colony (a) was used to gauge the extent of the AP staining (b). Both images were taken at 5x magnification.

Karyotype Analysis

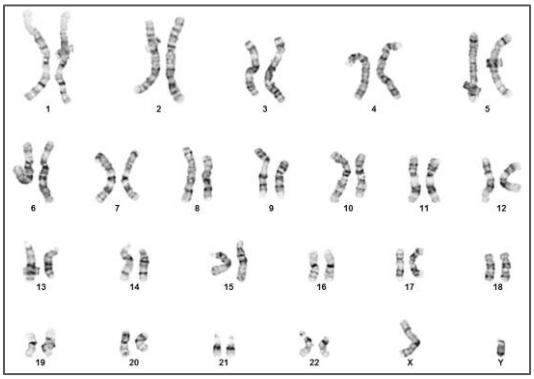
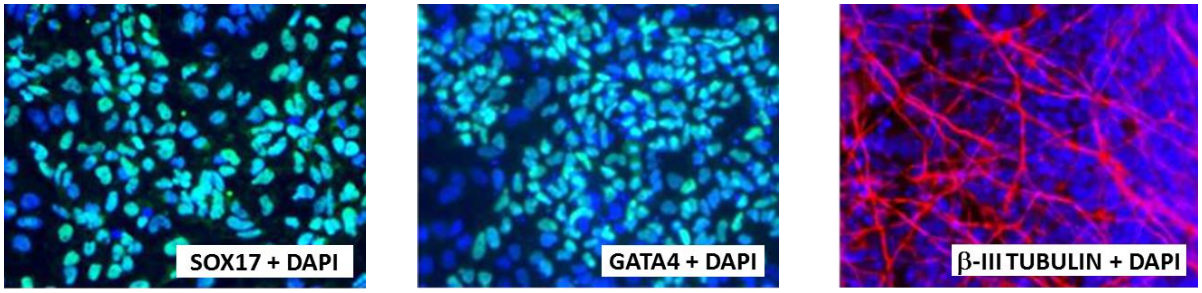


Figure 2. Karyotype analysis to rule out genetic aberrations. This iPSC line demonstrates a normal male karyotype. Cytogenic analysis was performed on twenty G-banded metaphase cells from human iPSC line, ASE-9211 at passage 15. Nineteen cells demonstrated an apparently normal male karyotype, and one cell demonstrated a non-clonal chromosome aberration, which is most likely an artifact of culture.

Directed Differentiation to the Three Germ Layers



EN: Duct (200x) ME: Cartilage (200x) EC: Pigment Cells (200x)

Figure 3. Direct differentiation of ASE-9211 p18 to three germ layers. Immunofluorescent staining for lineage-specific biomarkers of three germ layers after direct differentiation of control hiPSC line, ASE-9211. The ASE-9211 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). Images shows are co-localization of biomarker with DAPI. Endoderm (EN) marker: SOX17 (green); Mesoderm (ME) marker: GATA4 (green); Ectoderm (EC) marker: β-III Tubulin (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-9211 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.