Applied StemCell, Inc.
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Datasheet

Human Induced Pluripotent Stem Cells Research Grade (from CD34+ Umbilical Cord Blood Cells; Male)

Product Information

Catalog Number	**ASE-9250**
Description	Applied StemCell, Inc. provides Human Induced Pluripotent Stem (hiPS) Cells (Research Grade) that were generated from CD34+ umbilical cord blood cells using an epsiomal reprogramming method. The ASE-9250 research grade hiPSC line is from a male donor, and has been fully characterized. This includes expression of pluripotency markers (Oct4, Sox2, Nanog, SSEA-4, and Tra-1-60; Figure 2), alkaline phosphatase (AP) staining (Figure 3), STR analysis (Figure 5), and the ability to differentiate into the three germ layers (Figure 4). The ASE-9250 human iPSC line can be used for CRISPR/Cas9 genome editing and differentiation to somatic lineages *in vitro*.
Reprogramming Method	Episomal
Tissue	CD34+ Umbilical Cord Blood Cells
Sex	Male
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	Each lot of human iPS cells has been tested for viability following recovery from cryopreservation. In addition, the hiPSCs have also been tested for functional pluripotency (formation of the three germ layers), expression of pluripotency markers (Oct4, Sox2, Nanog, SSEA-4, and Tra-1-60), presence of alkaline phosphatase (AP), STR, sterility, and for the absence of mycoplasma and pathogens (CofA available upon request).
Safety Precaution	**PLEASE READ BEFORE HANDLING ANY FROZEN VIALS**. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. 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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Additionally, use Universal Precaution when handling all biological samples as potential biohazardous material.

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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 hiPSC Line ASE-9250

Morphology Images

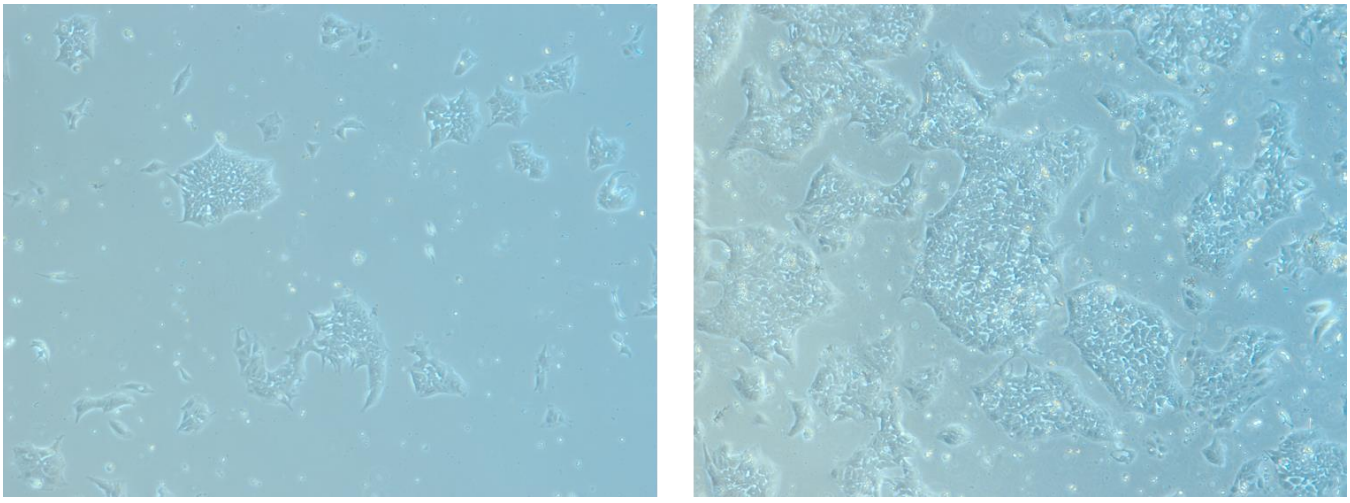

Figure 1. Morphology images of the ASE-9250 iPSCs captured 24 hours and 72 hours after thawing. The control human iPSCs were generated from normal CD34+ umbilical cord blood cells using an episomal method. The image of the hiPSCs on the left was taken 24 hours after thawing and the image on the right was capture 72 hours after thawing.

Expression of Pluripotency Markers

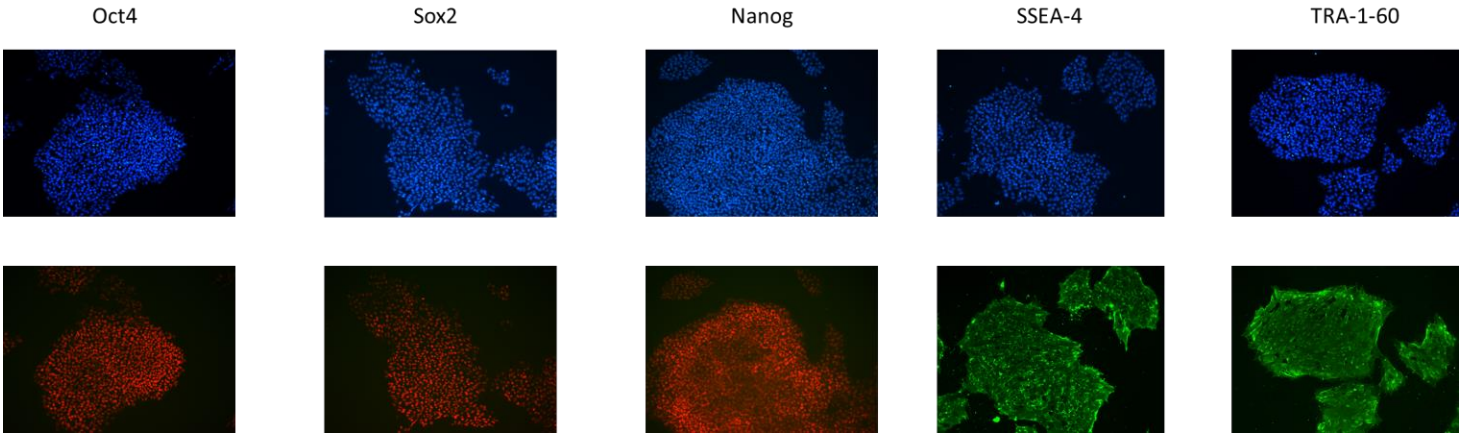

Figure 2. Expression of pluripotency markers. The ASE-9250 hiPSC line, derived from male CD34+ umbilical cord blood cells, expresses pluripotency markers Oct4, Sox2, Nanog, SSEA-4, and Tra-1-60 indicating pluripotency of the hiPSC line. The corresponding DAPI staining is above each image.

Alkaline Phosphatase (AP) Analysis

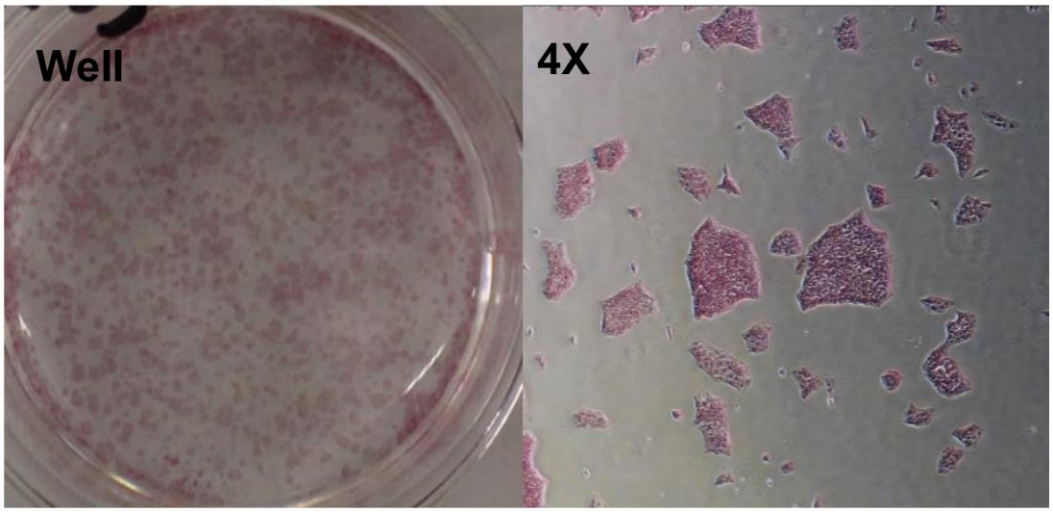

Figure 3. Alkaline phosphatase (AP) staining of ASE-9250. The ASE-9250 hiPSC line stained positive for alkaline phosphatase.

Directed Differentiation to the Three Germ Layers

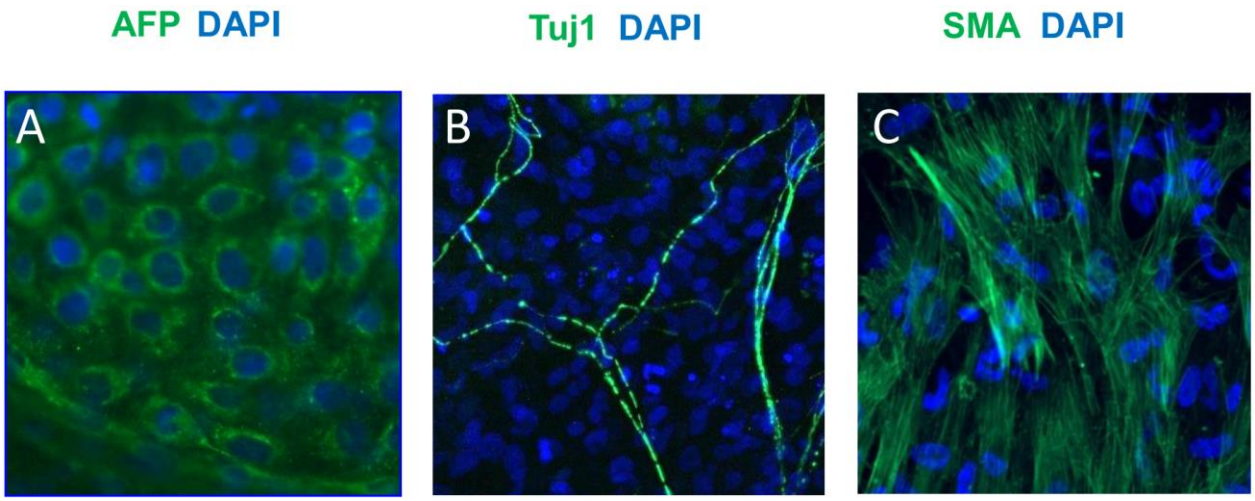

Figure 4. Directed differentiation of ASE-9250 to three germ layers. Immunofluorescent staining for three germ layers after directed differentiation of hiPSC line, ASE-9250. (A) Alpha-fetoprotein (AFP) for endoderm, (B) Neuron-specific class III β-tubulin (Tuj1) for ectoderm (C), Smooth Muscle Actin (SMA) for mesoderm.

STR Profile

Amelogenin	X	Y	D18S51	14	
vWA	16		Penta E	7	11
D8S1179	13	14	D5S818	11	
TPOX	8		D13S317	9	11
FGA	18	25	D7S820	10	11
D3S1358	15		D16S539	11	13
THO1	6	9.3	CSF1PO	12	
D21S11	31.2	33.2	Penta D	11	13

Figure 5. STR profiling of the ASE-9250 hiPSC line. The DNA STR profile of cell line ASE-9250 is consistent with the presence of a single cell line.

Materials Needed but not Provided

Material	Vendor	Catalog#
Serological Pipettes (1 mL, 5 mL, 10 mL and 25 mL)		
Sterile 15 mL and 50 mL Conical Tubes		
Pipette Tips		
9" Disposable Pasteur Pipettes		
Pasteur Pipette		
Culture vessels in different sizes (12-well-plate, 6-well-plate, 24 well plate)		
1.5 mL Eppendorf Tube		
Ice and ice box		
Cryo-tube		
Corning® CoolCell® Freezing System		
1x DPBS (without Calcium and Magnesium)	Life Technologies	14190-144
TesR™-AOF medium	Stemcell Technologies	100-0401
Vitronectin	ThermoFisher Scientific	A14700
Versene solution	ThermoFisher Scientific	15040066
CTS™ PSC Cryomedium	ThermoFisher Scientific	A4238801
RevitaCell™ Supplement (100x)	ThermoFisher Scientific	A2644501

Protocol

1. Coating Culture Vessels with Vitronectin

1.1. Upon receipt, thaw the vial of vitronectin at room temperature and prepare 60 µL aliquots of vitronectin in polypropylene tubes. Freeze the aliquots at –80°C or use immediately.

1.2. Prior to coating culture vessels, calculate the working concentration of vitronectin using the formula below and dilute the stock.

Note: Refer to the below table for culture surface area and volume required.

Culture vessel	Approximate surface area (cm2)	Diluted substrate volume (mL)
6-well plate	10 cm^2/well	1 mL/well
12-well plate	4 cm^2/well	0.4 mL/well
24-well plate	2 cm^2/well	0.2 mL/well
35-mm dish	10 cm^2	1 mL
60-mm dish	20 cm^2	2 mL
100-mm dish	60 cm^2	6 mL

1.3. A final coating concentration of 0.5 µg/cm^2 for human iPSC culture is recommended by the manufacturer.

Equations
Working Concentration = Coating concentration x (Culture surface area/Volume required for surface area)
Dilution Factor = (Stock concentration (0.5 mg/mL)) / (Working concentration)

Example: To coat a 6-well plate at a coating concentration of 0.5 µg/cm^2, you will need to prepare 6 mL of diluted vitronectin solution (10 cm^2 /well surface area and 1 mL of diluted vitronectin/well) at the following working concentration:

Working Concentration = 0.5 µg/cm^2 x (10 cm^2/ 1 mL) = 5 µg/mL

Dilution Factor = (0.5 mg/mL / 5 µg/mL) = 100x (i.e., 1:100 dilution)

1.4. To coat the wells of a 6-well plate, remove a 60 µL aliquot of vitronectin from –80°C storage and thaw at room temperature. You will need one 60 µL aliquot per 6-well plate.

1.5. Add 60 µL of thawed vitronectin into a 15-mL conical tube containing 6 mL of sterile DPBS without calcium and magnesium at room temperature. Gently resuspend by pipetting the vitronectin dilution up and down.

Note: This results in a working concentration of 5 µg/mL (i.e., a 1:100 dilution).

1.6. Aliquot 1 mL of diluted vitronectin solution to each well of a 6-well plate.

1.7. Incubate at room temperature for 1 hour.

Note: Dishes can now be used or stored at 2–8°C wrapped in laboratory film for up to a week. Do not allow the vessel to dry. Prior to use, pre-warm the culture vessel to room temperature for at least 1 hour.

1.8. Aspirate the diluted vitronectin solution from the culture vessel and discard. It is not necessary to rinse off the culture vessel after removal of vitronectin. Cells can be passaged directly onto the vitronectin-coated culture dish.

Note: Immediately after removal of the vitronectin solution, TeSR™ medium should be added to prevent drying of the culture wells.

2. Thawing iPS Cells

2.1. Remove one vial of ASE-9250 from liquid nitrogen tank storage, transfer the vial on dry-ice to the lab area.

2.2. Quickly thaw the iPSCs in a 37°C water bath until only a small frozen pellet remains.

2.3. Wipe the cryovial with a paper towel sprayed with 70% ethanol and place it into the hood.

2.4. Use a 5 mL serological pipette to transfer the cell suspension to a 50 mL conical tube.
2.5. Dilute the cell suspension with 3 mL of TeSR™-AOF (media without RevitaCell™), adding it dropwise while gently rocking the tube back and forth to avoid osmotic shock to the cells.
2.6. Centrifuge the cells for 4 minutes at 200 x g, at room temperature.
2.7. Aspirate off the medium and be careful not to disturb the pellet.
2.8. Gently resuspend the cells in TeSR™-AOF supplemented with RevitaCell™ Supplement at a 1X final concentration.
Example: 100 µL of RevitaCell™ Supplement in 10 mL of TeSR™-AOF medium.
Note: Do not add any additional rock inhibitor to the medium.

2.9. Gently pipette cells up and down to resuspend the cells and transfer 2 mL into each well of a vitronectin coated 6 well plate.
Note 1: The recommended lowest density that the iPSCs should be thawed at is 200,000 cells per well of a 6-well plate.
*Note 2: Count viable cells to control for issues with thawing (e.g., if thawing technique is too rough, too slow, etc., then an increased volume can be used to seed the right number of *living* cells per well).*

2.10. Place the plate in the 37°C incubator and move the plate back and forth and side to side twice to spread the clumps evenly in the well.
2.11. Incubate the cells in the incubator for 24 hours. Following incubation, aspirate the media with RevitaCell™ and replace it with 2 mL of fresh TeSR™-AOF **WITHOUT** RevitaCell™.

3. Passaging of Cells

3.1. Aspirate the medium from the iPSC culture.
3.2. Wash once with 1 mL of 1X DPBS (without calcium and magnesium).
3.3. Add 750 µL of 0.5 mM Versene EDTA to each well of a 6 well plate. Incubate the cells for 3-5 minutes at 37°C.
3.4. Observe the cells under the microscope. The cells at the edge of the colonies will start to separate and round up.
3.5. With the cells still attached, aspirate off the Versene EDTA and add 1 mL of TeSR™-AOF medium to the wells.
3.6. Pipette gently 3 to 4 times to detach the cells from the well.
Note: Do not pipette harsh or more times to prevent creation of single cells.

3.7. Transfer the desired dilution (usually around 1:3 – 1:10 depending on the growth rate of the individual cell line) to the wells of the new vitronectin coated plate with TeSR™-AOF.
*Note: RevitaCell™ should *not* be used every time the cells are passaged. When stem cells are kept as clumps, ROCK inhibitor is not needed. Constant use of ROCK inhibitors can increase differentiation, so it should be limited to situations when single cells are needed (i.e., have to count, do FACS, etc.), or when they are created by accident (i.e., treated with Versene for too long, pipetted too much, etc.).*

3.8. Place the plate in the CO2 incubator and move the plate back and forth and side to side twice to spread the clumps evenly in the well.

4. Cryopreservation

4.1. Aspirate the medium from the iPSC culture.
4.2. Wash once with 1 mL of 1X DPBS (without calcium and magnesium).
4.3. Add 750 µL of 0.5 mM Versene EDTA to each well of a 6 well plate. Incubate the cells for 3-5 minutes at 37°C.
4.4. Observe the cells under the microscope. The cells at the edge of the colonies will start to separate and round up.

4.5. With the cells still attached, aspirate off the Versene and add 1 mL of CTS™ PSC Cryomedium to the wells. Pipette gently 3 to 4 times to detach the cells from the well.

4.6. Dispense aliquots of the suspension into cryogenic vials 1 mL to 1.5 mL in a 2-mL cryovial.

Note: Mix the cell suspension in CTS™ PSC Cryomedium frequently to maintain a homogenous suspension. Mix the cell suspension by gentle inversion to prevent breaking cells into smaller clumps.

4.7. After aliquoting, move the vials to a Corning® CoolCell® Freezing System and place the CoolCell® holder to a -80°C freezer.

4.8. After one day or two, transfer frozen vials to an LN2 tank.