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

Datasheet

Genome Edited iPSCs DISC1$^{-/-}$ Knockout

Product Information

Catalog Number	**ASE-9406ASC**
Description	Applied StemCell's Genome Edited series of iPSC lines are ideal as *in vitro* models for neurodegenerative diseases such as Parkinson's Disease, Alzheimer's Disease, ALS, Autism and more. The ASE-9406ASC iPSC line is engineered with a bi-allelic (homozygous) knockout (KO) of the DISC1 gene (DISC1$^{-/-}$) that has been implicated in the development of Schizophrenia. The parental iPSC line, ASE-9211 is an integration-free, normal karyotype iPSC line derived from male fibroblast. The DISC1 knockout line can be further differentiated into an isogenic panel of neurons and glia for disease modeling and drug/toxicity screening applications.
Amount	1x10^6 cells/vial
Parental Cell Line	ASE-9211 (Male; Control iPSC)
Gene Knockout	DISC1
Generated KO Line	DISC1 KO, Clone E1
Type of KO	Bi-allelic (homozygous)
Cell Passage #	20
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, SSEA-4, and Tra-1-81; and for the absence of bacteria, fungi, mycoplasma (CoA 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.
Restricted Use	This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

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

Characterization of the Genome Edited iPSC Line ASE-9406ASC

Morphology Image

Figure 1. Morphology image of the iPSC DISC $^{-/-}$ knockout line (Clone E1). The DISC1$^{-/-}$ knockout iPS cell line was generated using the CRISPR/Cas9 platform.

Expression on Pluripotency Markers

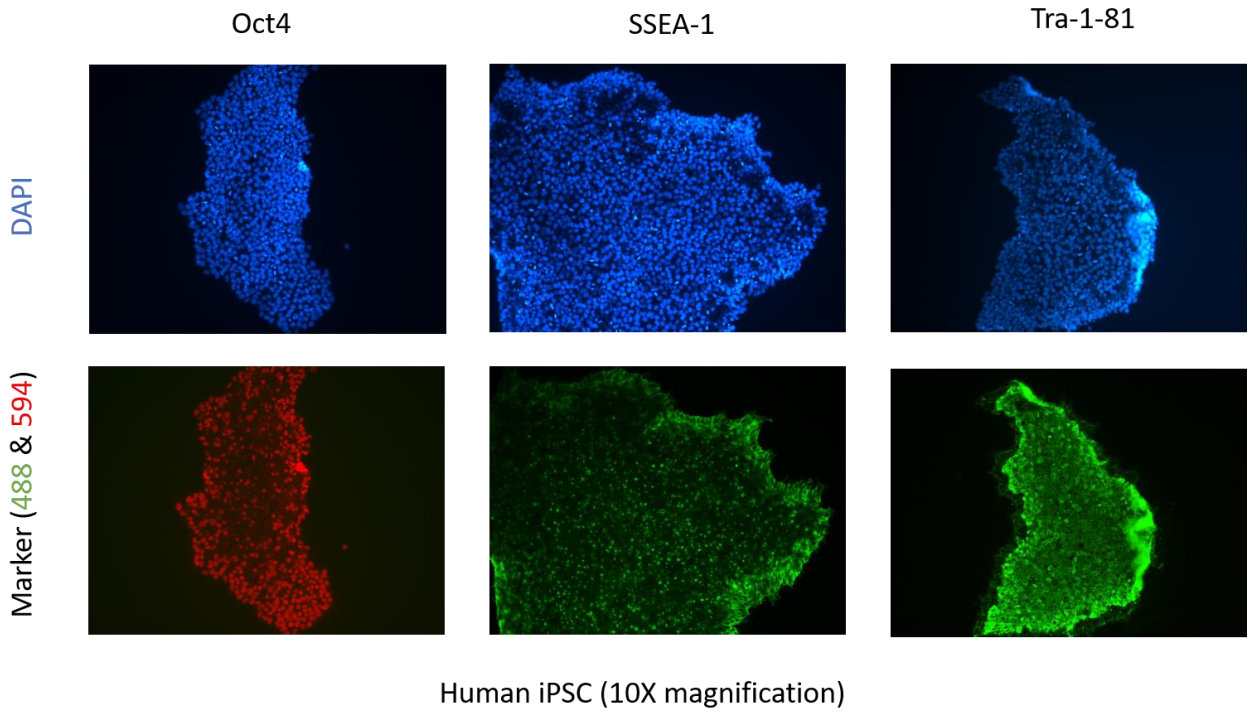

Figure 2. Expression of pluripotency markers in the DISC1$^{-/-}$ iPSC line. The homozygous knockout iPSC line, DISC1$^{-/-}$, expresses pluripotency markers Oct4, SSEA-1, and Tra-1-81, indicating pluripotency of the iPSC line after genome editing. Nucleus stained with DAPI (blue).

Genotyping

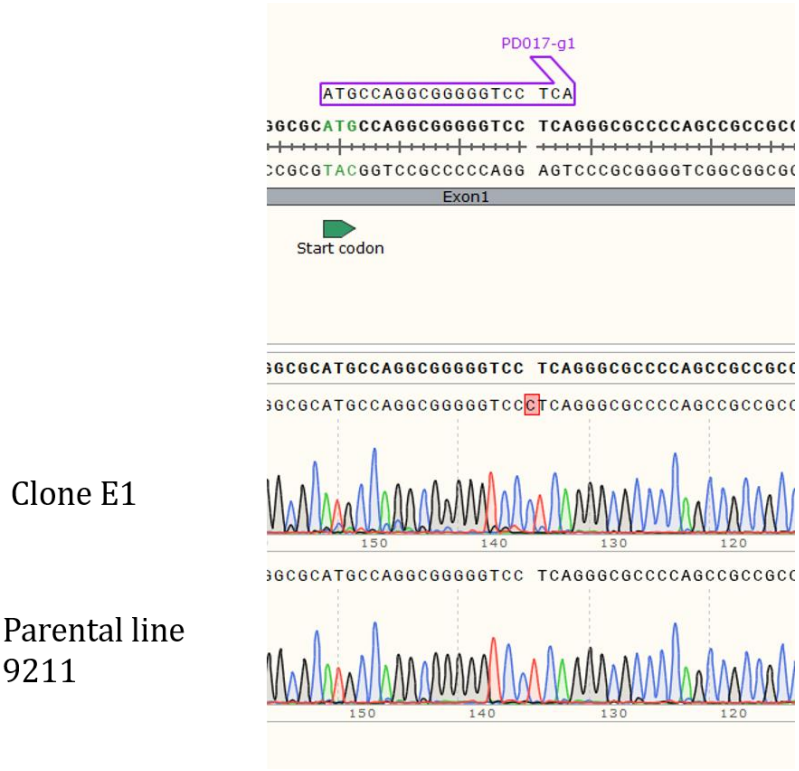

Figure 3. Genotyping of the DISC1$^{-/-}$ KO iPS cell line (Clone E1). Sequencing chromatogram of the KO iPSC line with 1 bp insertion in Exon 1 in DISC1 (top: Clone E1) compared to the wild type (WT) parental line, ASE-9211 (bottom).

Additional Reagents Required but not Provided

- Rock Inhibitor, Stemgent, Cat# ASE-04-0012
- mTeSR™ Plus Feeder Free Media, STEMCELL Technologies, Cat# 05825
- BD Matrigel™ hESC-qualified Matrix Features, BD Biosciences, Cat# 354277
- DMEM, Life Technologies, Cat# 11995081
- 0.5mM EDTA/PBS, Life Technologies, Cat# 15575-020
- CryoStor® CS10 freeze Medium, Stemcell technology, Cat# 210102

Equipment Required

Equipment	Specifications
Centrifuge	(e.g. Thermo Centra CL2)
Conical Tubes	Polystyrene Conical Tube 15 mL, Corning Inc., Cat # 430790 Polystyrene Conical Tube 50 mL, Corning Inc., Cat# 176740
Cell Counter/Hemocytometer	
Cell Culture Dishes	60mm/15mm, Corning Inc., Cat# 430196

Protocol

All steps must be performed according to standards required for sterile cell culture work.

1. Handling Upon Receiving

Applied StemCell's Genome Edited iPSCs are shipped on dry ice at ambient temperature. Single or multiple vials with cryopreserved cells are packed in a transparent bag, which is buried in dry ice. Upon receiving the product, check the integrity of the packages and the presence of dry ice (contact Applied StemCell, if the integrity of a package has been compromised, e.g., no dry ice in the package).

Cells can be plated immediately after arrival or transferred into liquid nitrogen. Do not remove the vials from dry ice during transportation to storage units. Immediately transfer components (especially the cryopreserved iPSCs) to storage units, avoiding prolonged exposure to room temperature.

2. Coating Cell Culture Dishes or Flasks with BD Matrigel™ hESC-qualified Matrix Substrate

Cell culture vessels should be coated one day before or on the day of plating the cells. Please read producer's manual for handling BD Matrigel™ hESC-qualified Matrix.

Important producer's notes:

It is extremely important that BD Matrigel hESC- qualified Matrix and all culture ware or media coming in contact with Corning Matrigel™ hESC-qualified Matrix should be pre-chilled/ice-cold since BD Matrigel™ hESC-qualified Matrix will start to gel above 10°C.

The dilution is calculated for each lot based on the protein concentration. Prepare aliquots according to the dilution factor provided on the Certificate of Analysis (use 1.5-2.0 mL tubes). The volume of the aliquots is typically between 270-350 µL.

2.1 Pre-chill pipettes tips and dishes at 4°C.
2.2 Thaw an aliquot (typically between 270-350 µL) of BD Matrigel™ hESC-qualified Matrix at 4°C (approximately 45-60 minutes).
2.3 Transfer the aliquot on ice into biological safety cabinet.
2.4 Prepare a 25 mL aliquot of cold DMEM in 50 mL conical tube and keep on ice.
2.5 Using p1000 micropipette, transfer 1000 µL of the cold DMEM from the above tube into the tube with

Matrigel and mix up and down several times. Transfer the Matrigel solution to the 50 mL conical tube containing cold DMEM and mix several times with a serological pipette (keep on ice).

2.6 Immediately, coat pre-chilled 6-well culture dishes with Matrigel/DMEM solution (1 mL per well).

2.7 Distribute coating matrix evenly and incubate at room temperature (15-25°C) for at least 1 hour before use.

2.8 Coated dishes can be used immediately or can be stored at 4°C for up to 7 days (aseptic conditions).

Table 1. Recommended volumes of coating reagents for various vessels.

Vessel	Approx. Surface Area	Matrigel
96 well plate	0.33 cm^2/well	50 µL/well
4 or 24 well plate	2 cm^2/well	250 µL/well
35 mm dish	10 cm^2	1.5 mL
60 mm dish	20 cm^2	2.5 mL

3. Recovering iPSCs from Frozen Stock (Feeder free, Single Cell Suspension)

3.1 Pre-warm mTeSR™ Plus culture medium to 37°C and supplement the medium with 10 µm (final concentration of ROCK inhibitor).

3.2 Pre-warm Matrigel coated dishes.

3.3 Pipette out 5 mL of the above medium into a 15 mL conical tube.

3.4 Transfer a vial with frozen iPSCs (on dry ice) to the operation side and thaw cells immediately in 37°C degree water bath until only a small piece of ice is still visible (approximately 1-1.5 minutes).

3.5 Transfer the vial to the biological safety cabinet and transfer thawed iPSCs slowly and drop-wise into a 15 mL conical tube containing 5 mL of pre-warmed mTeSR™ Plus + ROCK medium under constant swirling.

3.6 Wash the cryo-vial with additional 1 mL of medium and transfer to the same 15 mL tube.

3.7 Centrifuge cells at 200g for 5 minutes.

3.8 Aspirate supernatant carefully without disturbing the pelleted cells and re-suspend the pellet carefully but thoroughly (5-6 up and down mixes) in 5 mL of fresh mTeSR™ Plus + ROCK medium using 5 mL serological or p1000 micro-pipette with a long tip.

3.9 Aspirate Matrigel solution from coated dishes and immediately seed the iPSCs into 2 wells of coated 6-well plate (do not allow the matrix to dry out).

Optional: *Aspirate Matrigel before the cells are thawed and add enough medium to cover the bottom of the culture dish.*

3.10 Distribute cells evenly by performing cross-like moves and place dish(es) in 37°C / 5°CO_2 cell culture incubator.

3.11 Change mTeSR™ Plus medium (without ROCK inhibitor) every day.

4. Passage of Feeder Free Cultured iPSC Using EDTA/PBS

4.1 Pre-warm plates coated with Matrigel at 37°C.

4.2 Prepare new plate for transfer of clones: Remove the Matrigel solution from the plates and add appropriate volume of pre-warmed mTeSR™ Plus supplemented with 10 µM ROCK inhibitor to each plate.

4.3 Aspirate media from the plate to be passaged. Add EDTA/PBS to each plate (1mL for 1 well of 6-well plate) and place the plate in a 37°C incubator for 3 minutes. Observe the cells under the microscope. The cells at the edge of the colonies will start to separate and round up.

4.4 With the cells are still attached, aspirate the EDTA and add 1 mL of mTeSR™ Plus medium.

4.5 Scrape the cells from the bottom of the well until all the colonies are floating; pipette up and down 2-3

times to break the colonies into small clumps.

4.6 Make a 1:10 dilution and transfer the cells to the wells of the new Matrigel® coated plates.

4.7 Place the plate in the CO_2 incubator and move the plate back-and-forth and side-to-side twice to spread the clumps evenly in the wells.

5. Cryopreservation of iPSCs

5.1 Label the cryovials as needed, based on 2 vials per well of a 6-well plate, and pre-chill them in a 4°C freezer.

5.2 Aspirate the medium from the hiPSC culture.

5.3 Wash once with 1 mL of 0.5 mM EDTA (in PBS).

5.4 Aspirate the PBS and add 1 mL of 0.5 mM EDTA per well in 6-well dish and incubate the cells for 3 minutes in a 37°C incubator.

5.5 Observe the cells under microscope until the cells at the edge of the colonies start to separate and round up.

5.6 With the cells still attached, aspirate the EDTA and add 2 mL of CryoStor® CS10 medium.

5.7 Scrape the cells from the bottom of the well until the colonies are all floating.

5.8 Aliquot the cell suspension in 2 pre-chilled and labeled cryovials: 1 mL in each vial.

5.9 Place the cryovials in a CoolCell® Freezing Container or in a Styrofoam rack at -80°C overnight, and transfer to liquid nitrogen the next day.