Applied StemCell, Inc.
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Datasheet

Human Induced Pluripotent Stem Cells (Amyotrophic Lateral Sclerosis Type 8)

Product Information

Catalog Number **ASE-9043**

Description Induced Pluriopotent Stem Cells (iPSCs) are a type of pluripotent stem cells that can be derived directly from adult somatic cells[1]. The derived iPSCs can propagate indefinitely, as well as, give rise to other cell types in the body. They provide an unlimited source of materials to develop *in vitro* preclinical models of human biology and diseases. iPSCs also hold great promise in the field of regenerative medicine by representing a single source of cells that could be used to replace those damaged/diseased cells. Applied StemCell iPSC catalog now includes human iPSC lines derived from the dermal fibroblasts of patients and their non-affected siblings with amyotrophic lateral sclerosis type 8 (ALS8; OMIM# 60827), a familial-type autosomal dominant degenerative motor neuron disorder with a P56S mutation in the VAPB gene[2]. These iPS cells are established from a single clone and expanded in feeder-free conditions. This series of ALS8 iPSC lines are useful as an early diagnostic tool for disease modeling, and for drug target discovery and cell therapy. ASC's iPSC catalog also includes normal human iPS cell lines as separate products (Catalog #ASE-9203/ 9109/ 9110). We also provide custom iPSC generation and iPSC differentiation services to meet your research needs.

Passage 6

Age 39 yr

Sex Female

Clinical information ALS8 (Family II)
Stage/ symptoms: Initial symptoms & spasticity
Symptoms onset time until collection: 1.5 years

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.

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

Media and Material Required but not Provided

iPSC Media and Materials for Feeder-free Culture System

- TeSR-E8 medium, StemCell Technologies, Cat# 05940
- Rock Inhibitor, Stemgent, Cat# ASE-04-0012
- BD Matrigel™ hESC-qualified Matrix Features, BD Biosciences, Cat# 354277; or any synthetic basement matrix
- ES-Sure™ FBS, Applied StemCell, ASM-5017
- 1X PBS, ThermoFisher, Cat# 14190144
- 0.5 M EDTA, ThermoFisher, Cat# 15575020
- DMSO, Sigma-Aldrich, Cat# D8418
- Cell Scraper, Corning, Cat# 3010

Protocol

1. Thawing human iPSCs using a feeder-free protocol

1.1 Prepare 2.5 mL of TeSR-E8 medium + 10 µM Rock Inhibitor.
1.2 Aspirate the medium from a well of a 6-well plate; wash once with 1x PBS, and add 1 mL of TeSR-E8 medium + Rock Inhibitor. Label the plate with the name of the clone, passage number and date, and place it in the incubator.
1.3 Bring the cryovial on dry ice to the tissue culture room.
1.4 Quickly thaw the hiPSCs in a 37°C water bath by gently shaking the cryovial continuously until only a small frozen pellet remains.
1.5 Wipe the cryovial with a Kimwipe sprayed with 70% ethanol and place it into a biosafety cabinet.
1.6 Transfer the cells to a 50 mL conical tube.
1.7 Add 5 mL of cold TeSR-E8 medium dropwise while swirling the conical tube with the cells.
1.8 Centrifuge the cells for 5 min at 300g at 4°C.
1.9 Aspirate off the medium and add 1mL of TeSR-E8 medium + Rock Inhibitor.
1.10 Gently flick the conical tube to resuspend the cells and transfer them to the well of a Matrigel™ plate using a 5 mL serological pipette.
Note: Prepare Matrigel plates the previous day and not more than 3 days prior to thawing.
1.11 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 well.
1.12 Change medium daily. Usually, after 1-2 weeks the cells are ready to be split.

2. Passaging/ splitting human iPSCs using EDTA

2.1 Aspirate the medium from the hiPSC culture.
2.2 Wash once with 2 mL of 1x PBS.
2.3 Add 1 mL of 0.5 mM EDTA (in PBS) per well of a 6-well plate and incubate the cells for 3-5 min at RT.
2.4 Observe the cells under a microscope. When the cells at the edge of the colonies start separating and round up, aspirate the EDTA and add 1 mL of TeSR-E8 medium (or any medium for feeder-free culture of hiPSCs).
2.5 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.
2.6 Transfer the desired dilution to the wells of the new Matrigel™-coated plate (usually at 1:3 – 1:10 ratio).
Note: Prepare Matrigel plates (or any other basement matrix) not more than a week prior to passaging cells
2.7 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 well.

Note: hiPSCs are passaged as clumps of 50-200 cells, rather than single cells. On average, cells need to be passaged once a week before the colonies are large enough to merge with one another.

3. Cryopreserving human iPS cells

3.1 Label the cryovials as needed, based on1 vial per well of a 6-well plate, and pre-chill them in a – 20°C freezer.
3.2 Aspirate the medium from the hiPSC culture.
3.3 Wash once with 2 mL of 1x PBS.
3.4 Add 1 mL of 0.5 mM EDTA (in PBS) per well of a 6-well plate and incubate the cells for 3-5 min at RT.
3.5 Observe the cells under a microscope. When the cells at the edge of the colonies start separating and round up, aspirate the EDTA and add 0.5 mL of cold TeSR-E8 medium.
3.6 Scrape the cells from the bottom of the well until the colonies are all floating.
Note: Do not pipette up and down when freezing hiPSCs.
3.7 Add 0.5 mL of cold 2x freezing medium dropwise while mixing the cells in the plate.
Note: Cold 2x Freezing medium (60% FBS, 20% TeSR-E8 medium, 20% DMSO)
3.8 Place the total 1 mL mixture of cells+medium in a cold cryovial.
3.9 Place the cryovial in a MrFrosty container or in a Styrofoam rack at - 80°C overnight and transfer to liquid nitrogen the next day.
Note: There may be 5-20% differentiated cells after thaw. The cells will be stabilized after 2-3 passages.

References

1. Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., ... & Shibata, T. (2011). A more efficient method to generate integration-free human iPS cells. Nature methods, 8(5), 409-412.
2. Mitne-Neto, M., Machado-Costa, M., Marchetto, M. C., Bengtson, M. H., Joazeiro, C. A., Tsuda, H., ... & Muotri, A. R. (2011). Downregulation of VAPB expression in motor neurons derived from induced pluripotent stem cells of ALS8 patients. Human molecular genetics, 20(18), 3642-3652.