



Human Induced Pluripotent Stem Cells (Parkinson's Disease; Fibroblasts)

Product Information

Catalog Number ASE-9208

Description Induced Pluripotent 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. iPS cells, thus, 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 is proud to offer human iPS cell lines derived from the human somatic cells (dermal fibroblasts, adipose-derived stem cells, peripheral blood mononuclear cells) from patients with Parkinson's Disease (PD). The pertinent donor information is available upon request. These iPS cells are established from a single clone and expanded in feeder-free conditions. Normal human iPS cell lines are also available as separate products (Catalog #ASE-9203). We also provide custom iPSC generation and iPSC differentiation services to meet your needs.

Tissue Fibroblasts

Age 82 yr

Sex Female

Clinical information Parkinson's disease

Quantity 0.5-1 x 10⁶ 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 The cells have been fully characterized for their pluripotency and self-renewal (Figures 1-5). All the cells provided are negative for mycoplasma, bacteria, yeast, and fungi. HIV-1, hepatitis B and hepatitis C. A Certificate of Analysis is provided for each cell lot purchased.

Viability: >90%

Purity by morphology and antibody staining: >90% undifferentiated cells (by morphology), 1 week post-thaw

G-banding Karyotyping (see report): Normal

Safety Precaution **PLEASE READ BEFORE HANDLING ANY FROZEN VIALS.** Please wear appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles)

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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.

Pluripotency Marker Staining

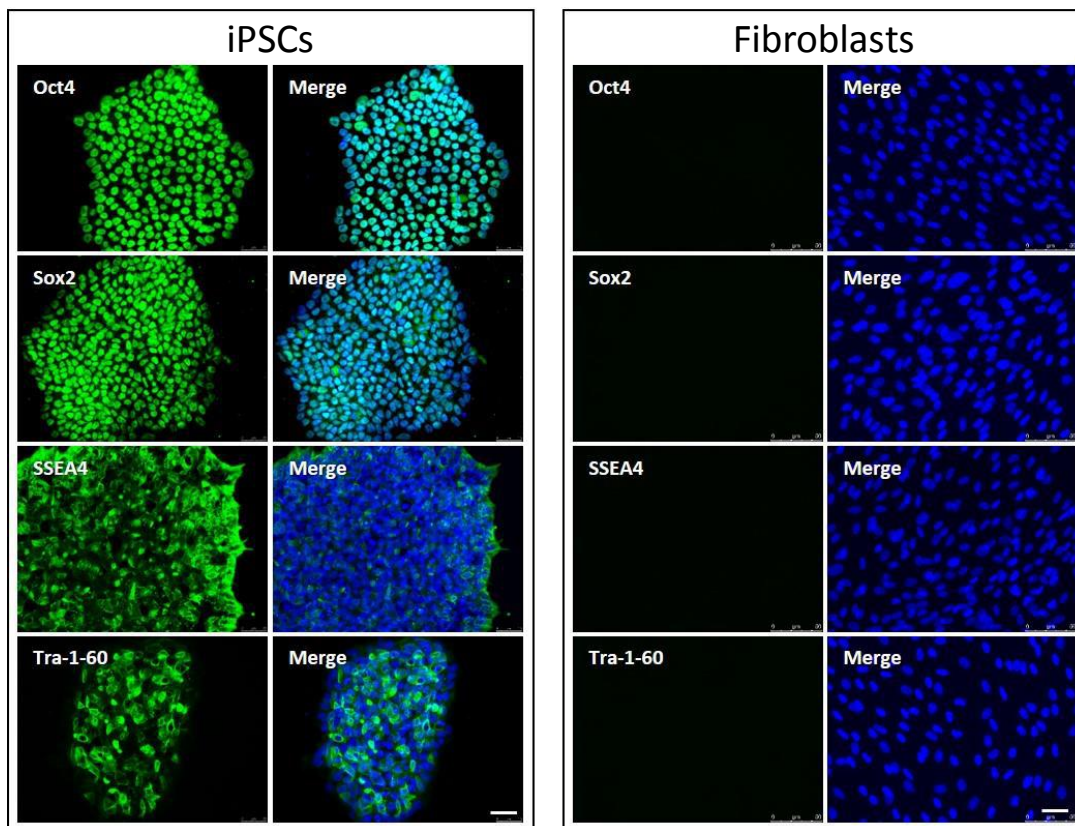


Figure 1. The iPSC line was maintained in Essential 8 medium on Geltrex-coated plates. The cells were stained with the pluripotency markers, listed above. Scale bars, 50 μ m.

Embryoid Body Formation Assay

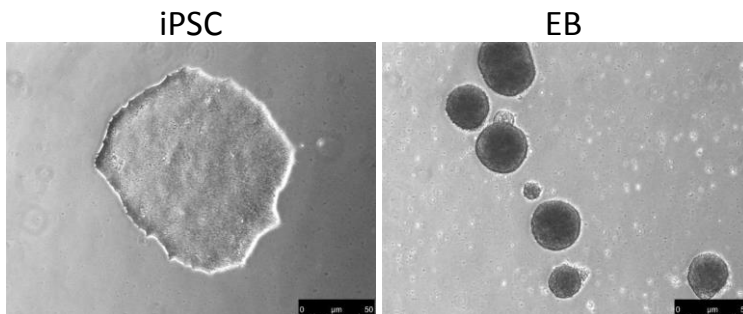


Figure 2. The iPSC line was maintained in Essential 8 medium on Geltrex-coated plates. The cells were subsequently maintained in Essential 6 medium in ultra-low attachment plates to form Embryoid Bodies (EBs). Scale bars: 50 μ m.

EB Germline Marker Staining

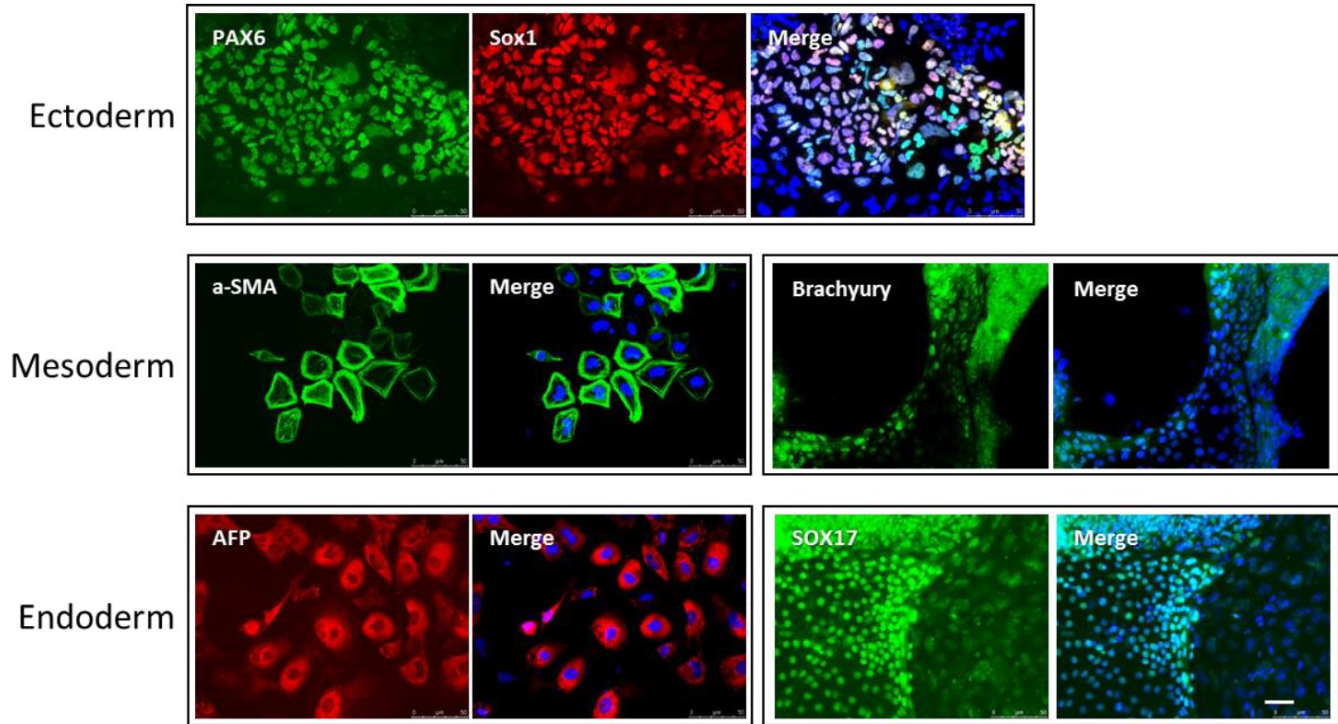


Figure 3. The iPSC line was maintained in Essential 8 medium on Geltrex-coated plates. The cells were subsequently maintained in Essential 6 medium in ultra-low attachment plates to form Embryoid Bodies (EBs) for seven days. The EBs were re-attached to gelatin-coated plates in Essential 6 medium for seven to fourteen days before antibody staining.

Pluripotency Marker RT-PCR

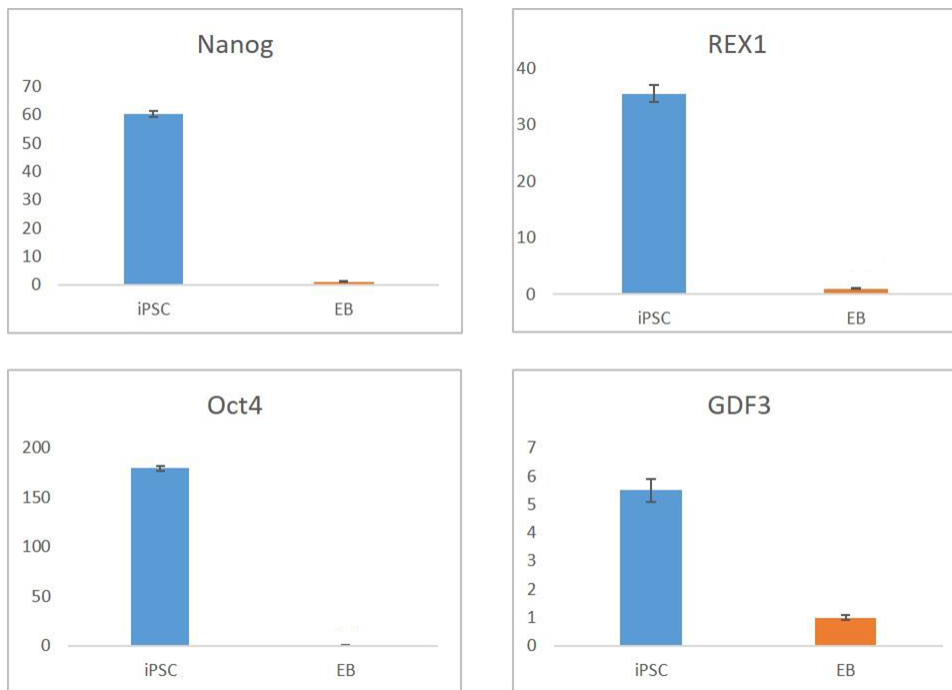


Figure 4. Relative gene expression in iPSCs and EBs. The y-axis represents gene expression level normalized to NAT-1, which was used as an internal control. The iPSC line was maintained in Essential 8 medium on Geltrex-coated plates. For embryoid body (EB) formation, the cells were maintained in Essential 6 medium in ultra-low attachment plates for seven days. The EBs were re-attached to Geltrex-coated plates in Essential 6 medium for seven to fourteen days before RT-PCR. Number of replicates = 3. Error bars represent +/- one SD.

G-Banding Karyotype Analysis Report



Banding Technique: GTL
Band Resolution: Good
Result: 46,XX
Cells Analyzed: 20

Figure 5. Karyotype analysis to rule out genetic aberrations. Cytogenetic analysis was performed on twenty (20) G-banded metaphase cells from the human iPSC line, and all twenty cells displayed an apparently normal female karyotype.

Protocol

Thawing of frozen cells

1. Upon receipt of the frozen cells, it is recommended to thaw the cells and initiate the culture immediately in order to retain the highest cell viability.
2. Prepare Matrigel™ coated plates or MEF feeder coated plates the day before recovering the cells.
3. To thaw the cells, put the vial in 37°C water bath with gentle agitation for ~1 minute. Keep the cap out of water to minimize the risk of contamination.
4. Pipette the cells into a 15 mL conical tube with 5 mL fresh culture media: Human iPSC Growth Medium can be used in on-feeder culture system, MEF Conditioned Medium or Human iPSC Feeder-Free Growth Medium can be used in feeder-free culture system.
5. Centrifuge at 50 g for 5 minutes at room temperature.
6. Remove the supernatant and re-suspend the cells in culture media supplemented with 10µM Y27632.
7. Seed the cells on Matrigel™ coated plates for feeder-free culture, or on feeder plates for on-feeder culture.
8. Incubate in 37°C CO₂ incubator overnight.
9. The next day, change to media without Y27632.
10. Change media daily until the cells are ready to be passaged. It may take 1-2 weeks to fully recover the cells before passaging.

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, Hong H, Nakagawa M, Tanabe K, Tezuka K, Shibata T, Kunisada T, Takahashi M, Takahashi J, Saji H, Yamanaka S. A more efficient method to generate integration-free human iPS cells. Nat Methods. 2011 May; 8(5):409-12.