Applied StemCell, Inc.
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Datasheet

Human iPSC-Derived Neural Stem Cells (Amyotrophic Lateral Sclerosis; Fibroblasts)

Product Information

Catalog Number **ASE-9026**

Description Neural stem cells (NSCs) are self-renewing, multipotent cells that generate the main phenotype of the nervous system. They primarily differentiate into neurons, astrocytes, and oligodendrocytes [1]. The recent discovery of induced pluripotent stem cells (iPSCs) not only overcomes the ethical and logistical issues associated with human embryonic stem cells, but also provides a flexible platform for generating various differentiated cell types from diseased individuals. iPSC-derived NSCs are a potentially valuable source of *in vitro* models for complex, polygenic human diseases, and are potentially useful for drug discovery and cell-based therapy applications [2].
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Applied StemCell provides high quality human NSCs derived from normal or diseased iPS cell lines. These cells express typical markers of neural stem and progenitor cells, e.g. NESTIN, PAX6 and SOX1 (Figure 1 and Figure 2), with the purity higher than 97% (Figure 3). The cells have been fully characterized for their self-renewal and multi-potency. The iPSC-derived NSCs can be differentiated into astrocytes or motor neurons (Figure 4).

All the cells provided by Applied StemCell are negative for mycoplasma, bacteria, yeast, and fungi. HIV-1, hepatitis B and hepatitis C. The basic donor information (gender / age / race) is provided for each cell lot purchased.

Species *Homo Sapiens*

Tissue Dermal skin (fibroblasts)

Age 50-55 yr

Sex Female

Race Caucasian

Clinical information Amyotrophic Lateral Sclerosis; Sporadic

Quantity 2 x 10^6 cells/vial

Growth Properties Adherent

Quality Control Each lot of human iPS cells has been tested for growth and viability following recovery from cryopreservation. In addition, each lot has been tested for expression of NSC markers (NESTIN, PAX6, and SOX1; Figures 1 and 2) with purity > 97% (Figure 3), and for its aibility to differentiate into astrocytes and neurons (Figure 4). These cells have also been tested for the absence of mycoplasma and pathogens (CoA available upon request).

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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.

Characterization of Human iPSC-derived Neural Stem Cells

Immunocytochemical Analysis of NSC Markers

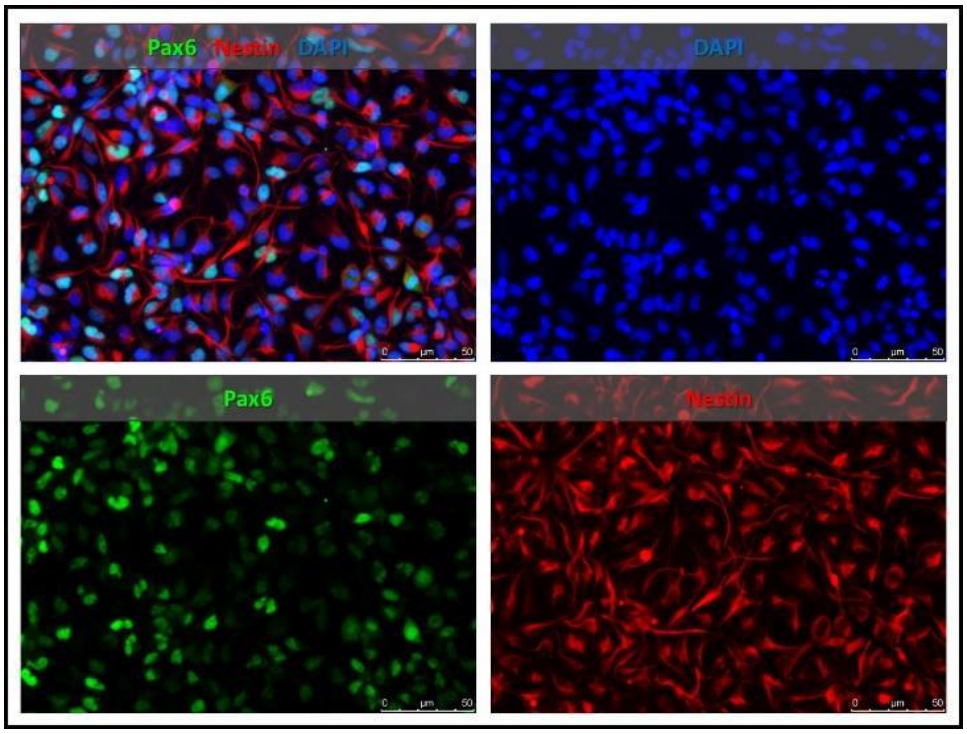

Figure 1. Immunohistochemical analysis for NSC markers NESTIN (red) and PAX6 (green). Nucleus staining with DAPI (blue).

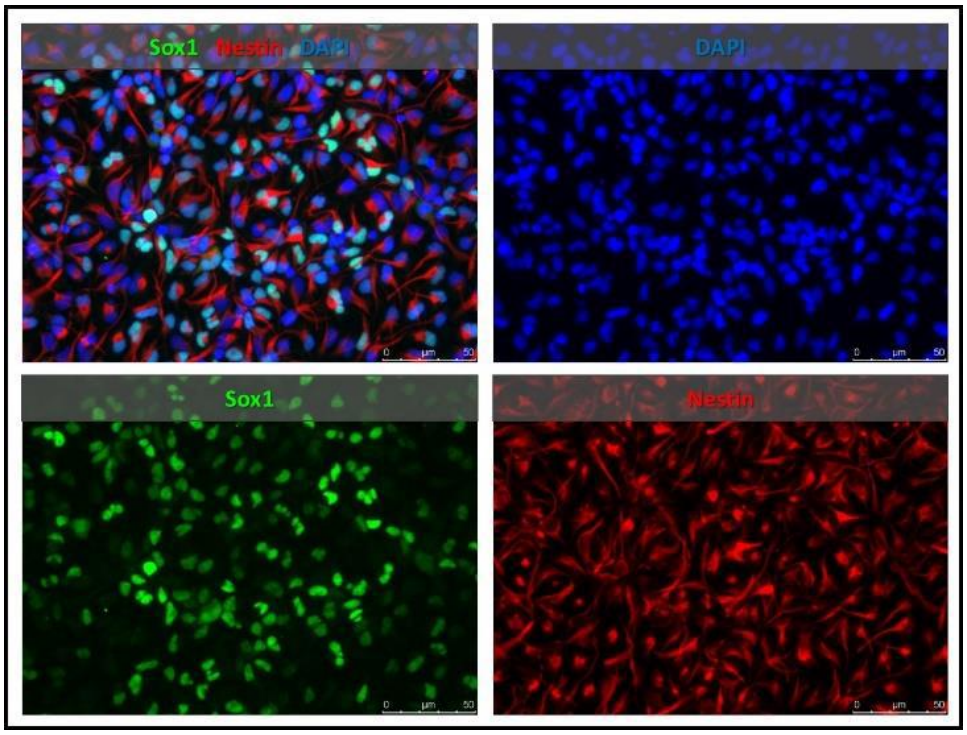

Figure 2. Immunohistochemical analysis for NSC markers NESTIN (red) and SOX1 (green). Nucleus staining with DAPI (blue)

Flow Cytometric Analysis

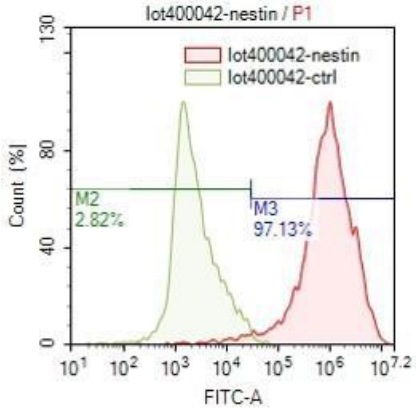

Figure 3. Flow cytometric analysis indicates more than 97% of the NSCs are Nestin positive.

Differentiation of NSCs into Astrocytes and Neurons

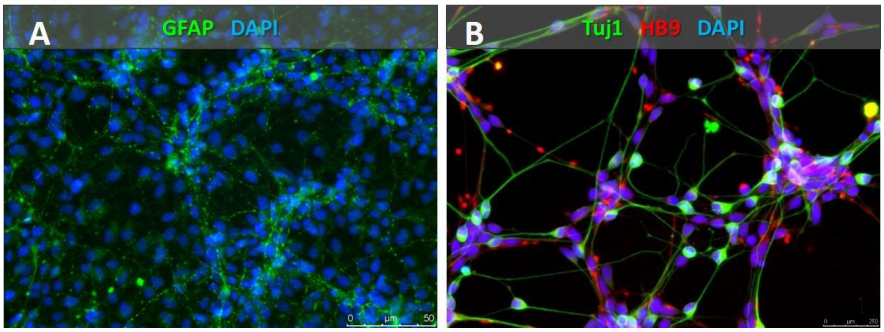

Figure 4. iPSC-derived NSCs can be differentiated into GFAP+ astrocyte (A) or HB9+ motor neurons (B).

Protocol

1. Thawing of Frozen Cells

1.1 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.
1.2 Prepare Poly-L-Ornithine/Laminin or Matrigel-coated plates the day before.
1.3 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.
1.4 Pipette the cells into a 15 mL conical tube with 5ml fresh human NSC culture media (ASM-4014/4015).
1.5 Centrifuge at 200g for 5 minutes at room temperature.
1.6 Remove the supernatant and re-suspend the cells in culture media.
1.7 Seed the cells on Poly-L-Ornithine/Laminin or Matrigel-coated plates.
1.8 Incubate in 37°C CO_2 incubator overnight.
1.9 Change media every other day until the cells are ready to be passaged. It may take 5-7 days to fully recover the cells before passaging.
1.10 The NSCs can be expanded for 3-5 passages and banked for future use. Please note that as the passage number increases, random differentiation may occur.

2. Subculture of NSCs

2.1 Prepare Poly-L-Ornithine/Laminin or Matrigel-coated plates the day before.
2.2 Remove the media from the cells.
2.3 Wash the cells once with D-PBS.
2.4 Add Accutase to the cells.
2.5 Incubate the cells in 37°C CO_2 incubator for 3-5 minutes.
2.6 Add two volumes of human NSC culture media (ASM-4014/4015).
2.7 Detach the cells by pipetting up and down several times.
2.8 Pipette the cells into a 15 mL conical tube.
2.9 Centrifuge at 200g for 5 minutes at room temperature.
2.10 Remove the supernatant and re-suspend the cells in human NSC culture media.
2.11 Seed the cells on Poly-L-Ornithine/Laminin or Matrigel-coated plates at desired density.

Reference

1. Alenzi, F; Bahkali, A (2011). "Stem cells: Biology and clinical potential". *African Journal of Biotechnology* 10 (86): 19929–40.
2. Dolmetsch R, Geschwind DH. (2011) “The human brain in a dish: the promise of iPSC-derived neurons”. *Cell*. 145(6):831-4.