7270 Trade Street, Suite 102, San Diego, CA 92121
Tel: (858)412-5988 Fax: (858)368-8716
Technical Supports: supports@ixcellsbiotech.com
Orders: orders@ixcellsbiotech.com

Product Information

Human iPS Cell Line (SOD1 mutation, A4V, HOM)

Catalog Number	30HU-101	Cell Number	~0.5-1 million cells/vial
Species	*Homo sapiens*	Storage Temperature	Liquid nitrogen

Product Description

Induced Pluripotent Stem Cells (iPSCs) are a type of stem cells reprogrammed from a multitude of somatic cells into an embryonic like pluripotent state. They have large self-renewal capability and can differentiate into any cell type from all three germ layers [1, 2]. Due to their high differentiation potential, iPSCs emerge as a promising cell model to promote cell differentiation for regeneration studies. Importantly, iPSCs reprogrammed from rare disease carriers can be subsequently expanded and differentiated indefinitely, allowing for genetically pertinent disease-specific iPSC model for research [3]. iPS cells, thus, are a unique model for studying a variety of processes that occur in the early development of mammals and are a promising tool in cell therapy of human diseases [4].

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that affects more than 18,000 people in the United States. 10% of the ALS cases are familial ALS with genetic causes. Mutations (over 150 identified to date) in the SOD1 gene have been linked to familial ALS [5-7]. The most frequent mutations are A4V and H46R. A4V (alanine at codon 4 changed to valine) is the most common ALS-causing mutation in the U.S. population, with approximately 50% of SOD1-ALS patients carrying the A4V mutation [8-10]. Approximately 10 percent of all U.S. familial ALS cases are caused by heterozygous A4V mutations in SOD1.

iXCells Biotechnologies is proud to offer human iPSCs with SOD1 A4V mutation. The cells were generated from a healthy donor iPS Cell Line (Cat# 30HU-002) by CRISPR/Cas9-based gene editing and contain HOM SOD1 A4V mutation (Fig. 1). The cells demonstrate hESC morphology, express the pluripotency markers (Oct4, Nanog, Tra-1-60 and Tra-1-81), and have normal karyotype. We also offer

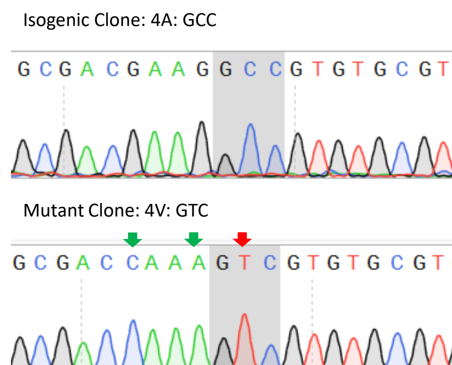

Figure 1. A4V mutation (red arrow) and two silent mutations (green arrows) have been introduced to SOD1 gene using CRISPR/Cas9 based genome editing technology. The targeted site is verified by genomic PCR/Sanger sequencing.

For Research Only All Rights Reserved iXCells Biotechnologies USA, Inc.

isogenic controls for this mutation (Cat# 30HU-101-ISO). Other cell types derived from the SOD1 A4V iPSC are also available (Motor Neuron: Cat# 40HU-101; Neural Stem Cells: Cat# 40HU-111; Skeletal Muscle Myoblasts: Cat#40HU-178). More disease-specific iPS lines are in development. We also provide custom iPSC generation and iPSC differentiation services to meet your needs.

Product Details

Tissue Origin	Human iPS Cell Line (SOD1 mutation, A4V, HOM)
Package Size	0.5~1.0 million cells/vial
Shipped	Cryopreserved
Storage	Liquid nitrogen
Growth Properties	Adherent
Media	Human iPSC Feeder-Free Growth Medium (Cat # MD-0019) Human iPSC Xeno-Free Growth Medium (Cat # MD-0074)

Protocols

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. Before recovering cells, prepare evenly coated Matrigel® / Cultrex® BME plates, following manufacturer's instructions.
3. To thaw the cells, put the vial in 37°C water bath with gentle agitation for ~1-2 minutes. 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 Feeder-Free Growth Medium** (Cat# MD-0019) or **Human iPSC Xeno-Free Growth Medium** (Cat# MD-0074) can be used in feeder-free culture system.
5. Centrifuge at 50-100*g* for 5 minutes at room temperature.
6. Remove the supernatant and re-suspend the cells gently in culture medium supplemented with 10 μM Y27632 (Cat# MD-0025).

Note: *Gently resuspend the cells to avoid formation of single cells.*

7. Seed the cells on Matrigel®/Cultrex® precoated plates for feeder-free culture.

Note*: It is recommended to seed the cells at three different densities in 6-well plates to ensure optimal recovery density.*

8. Incubate in 37°C CO_2 incubator overnight.
9. The next day, change media without Y27632.

 All Rights Reserved

10. Change media daily until cells are ready to be passaged. It may take 1-2 weeks (depending on the lot) to fully recover the cells before passaging.

Note*: There may be 5-20% differentiated cells after thaw. We recommend removing differentiated cells with a sterile tip or syringe at this point. The cells will be stabilized after 2-3 passages.*

Safety Precaution: *it is highly recommended that protective gloves and clothing should be used when handling frozen vials.*

Sub-Culturing Procedure for a 6-well Plate

1. Prepare evenly coated Matrigel®/Cultrex® plates according to manufacturers' instructions.
2. When the cells are 80-90% confluent, aspirate the medium and wash cells with 2 mL of sterile PBS/well.
3. Add 1 mL/well of ReLeSR™ and leave for 1-2 minutes at room temperature. Aspirate the ReLeSR and incubate the plate at 37°C for another 3-4 minutes.

Note*: Dissociation time may vary depending on the cell line used.*

4. Add 1 mL of medium and detach the colonies by gently tapping the plate or rocking side to side.
5. Transfer the detached cell aggregates to a 15 mL tube containing 5 mL fresh culture media.
6. Centrifuge at 50-100*g* for 5 minutes at room temperature and resuspend the pellet in desired volume. Avoid formation of single cells by gently resuspending the cell pellet.
7. Plate the cell aggregate mixture at the appropriate density onto pre-coated wells in recommended iXCells medium with 10 µM Y27632. The next day, change media without Y27632.
8. If the colonies are at an optimal density, cells will be confluent and ready to use in 4 - 7 days.

References

[1] Medvedev, S. P., Shevchenko, A. I., & Zakian, S. M. (2010). Induced Pluripotent Stem Cells: Problems and Advantages when Applying them in Regenerative Medicine. Acta naturae, 2(2), 18–28.

[2] Ghaedi, M., & Niklason, L. E. (2019). Human Pluripotent Stem Cells (iPSC) Generation, Culture, and Differentiation to Lung Progenitor Cells. Methods in molecular biology (Clifton, N.J.), 1576, 55–92.

[3] 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. (2011). A more efficient method to generate integration-free human iPS cells. Nature methods, 8(5), 409–412

[4] Ebert, A. D., Liang, P., & Wu, J. C. (2012). Induced pluripotent stem cells as a disease modeling and drug screening platform. Journal of cardiovascular pharmacology, 60(4), 408–416.

[5] Conwit RA (December 2006). "Preventing familial ALS: a clinical trial may be feasible but is an efficacy trial warranted?". Journal of the Neurological Sciences. 251 (1–2): 1–2.

[6] Al-Chalabi A, Leigh PN (August 2000). "Recent advances in amyotrophic lateral sclerosis". Current Opinion in Neurology. 13 (4): 397–405.

[7] Redler RL, Dokholyan NV (2012-01-01). "The complex molecular biology of amyotrophic lateral sclerosis (ALS)". Progress in Molecular Biology and Translational Science. Progress in Molecular Biology and Translational Science. 107: 215–62.

[8] Rosen DR, Bowling AC, Patterson D, Usdin TB, Sapp P, Mezey E, McKenna-Yasek D, O'Regan J, Rahmani Z, Ferrante RJ (June 1994). "A frequent ala 4 to val superoxide dismutase-1 mutation is associated with a rapidly progressive familial amyotrophic lateral sclerosis". Human Molecular Genetics. 3 (6): 981–7.

[9] Cudkowicz ME, McKenna-Yasek D, Sapp PE, Chin W, Geller B, Hayden DL, Schoenfeld DA, Hosler BA, Horvitz HR, Brown RH (February 1997). "Epidemiology of mutations in superoxide dismutase in amyotrophic lateral sclerosis". Annals of Neurology. 41 (2): 210–21.

[10] Valentine JS, Hart PJ (April 2003). "Misfolded CuZnSOD and amyotrophic lateral sclerosis". Proceedings of the National Academy of Sciences of the United States of America. 100 (7): 3617–22.

All Rights Reserved

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans. While iXCells Biotechnologies uses reasonable efforts to include accurate and up-to-date information on this product sheet, we make no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. iXCells Biotechnologies does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. iXCells Biotechnologies is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, iXCells Biotechnologies is not liable for damages arising from the misidentification or misrepresentation of cultures.
© iXCells Biotechnologies 2015. All rights reserved.