TARGATT™ Hypo plus hiPSC RUO (CD34+ Cord Blood Cells, Normal, Male)

The TARGATT™ Hypo plus hiPSC RUO is a hypoimmunogenic IPSC line.

The line is reprogrammed from CD34+ umbilical cord blood cells and rendered hypoimmunogenic by knocking out both the β2-microglobulin (B2M) gene and the HLA class II transactivator (CIITA) gene, eliminating cell-surface expression of HLA class I and class II molecules. Via TARGATT landing pad, five-gene immune-evasion cassette encoding HLA-F–B2M, CD200, CCL21, HLA-G1–B2M, and SERPINB9 was integrated into genome. This multiplex genome-engineered human iPSC platform is designed to reduce T-cell, natural killer (NK), and dendritic cell recognition, retain pluripotency, enable therapeutic gene expression, and support scalable manufacturing and development.

Catalog #	Product	Size
AST-9750	Human iPSCs from CD34+ Cord Blood, Male)	1.0×10^6 cells/vial

Parental Tissue Information	
Reprogramming Method	Episomal
Tissue	CD34+ umbilical cord blood cells
Sex	Male
Clinical Information	Normal

Intended Use

The AST-9750 human induced pluripotent cell line can be used for differentiation into somatic lineages *In vitro*.

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

Quality Assurance

Each lot of AST-9750 human iPSCs has been tested for viability following recovery from cryopreservation, morphology, expression of pluripotency markers (Oct-4, Sox-2, SSEA-4, TRA-1-60) and for normal male karyotype. Additionally, these iPSCs have been tested to confirm the absence of bacterial, fungal, and mycoplasma contamination.

All quality control test results are reported on a lot-specific Certificate of Analysis, available at appliedstemcell.com or upon request.

Storage Instructions and Stability

Store in liquid nitrogen freezer immediately upon receipt.

The iPSC line is stable for 6 months starting from the arrival date of the product. Every 6 months cells should be tested for pluripotency and differentiation potential to ensure they are viable for experimental use.

Precautions

The safety and efficacy of this product in diagnostic or other clinical uses have not been established. This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials while they are submerged in a liquid nitrogen freezer. During rapid 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. When retrieving the cell vial from a liquid nitrogen freezer, carefully twist open the cap to release any built-up nitrogen gas prior to thawing the cells.

Refer to the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Directions for Use

Required Materials (Not Provided)

Product	Vendor	Catalog#
mTeSR-Plus Medium	StemCell Technologies	05825
(Optional) 100 µg/mL Primocin™	Invivogen	ant-pm-2
Matrigel®	Corning	354277
Geltrax	ThermoFisher Scientific	A1413202
ROCK Inhibitor (Y-27632)	Sigma-Aldrich	SCM075
CloneR	StemCell Technologies	05889
CryoStor® CS10	StemCell Technologies	7930
0.5 M EDTA in PBS	ThermoFisher Scientific	15575-020
PBS	Life Technologies	14190136
Cell Scraper	VWR International	75799-938
Corning® CoolCell® FTS30	Corning®	432006

Protocol for Thawing human iPSCs

1. Prepare Matrigel® or Geltrex coated 6-well plates in advance, following vendor's instructions.

   Note: Prepare Matrigel or Geltrex plates the day before and not more than 3 days prior to thawing. Before transferring the cells, the Matrigel must be aspirated.

2. Prepare 6 mL of mTeSR-plus medium (mix of 4.8 mL basal medium and 1.2 mL 5x supplement) + Primocin (1:500 dilution) + 10 µM Rock Inhibitor.

   Note: Besides Rock inhibitor, the clone R can be used.

3. Add 1 mL of mTeSR-plus medium + Rock Inhibitor + Primocin in one well of a Matrigel®-coated plate. Prepare 1 well for each vial of frozen cells.
4. Bring the cryovial on dry ice to the tissue culture room.
5. Quickly thaw the iPSCs in a 37°C water bath, by gently shaking the cryovial continuously until only a small piece of ice remains.
6. Wipe the cryovial with a paper towel sprayed with 70% ethanol and place it into a biosafety cabinet.
7. Add 9 mL of mTeSR-plus medium + Primocin to a 15 mL conical tube.
8. Using a 1 mL pipette transfer the cells to the 15 mL conical tube dropwise while swirling the conical tube.
9. Centrifuge the cells for 3 minutes at 200 RCF and at room temperature.
10. Aspirate off the medium, and add 3 mL mTeSR-plus medium + Rock Inhibitor + Primocin.
11. Gently flick the conical tube to resuspend the cells and transfer them to the 1 well of the Matrigel® or Geltrex plate using a 5 mL serological pipette.
12. 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 wells.
13. Medium can be changed 2 days after thawing. After the 2 days, if the number of attached colonies is still low (less than 5% confluency), change half of the medium (aspirate 1 mL and add 1 mL of mTeSR-plus medium + Primocin).
14. Once colonies are stabilized, change the medium daily. Usually, within 1 week the cells are ready to be split.

15. When the AST-9750 hiPSC colonies are big or close enough to merge, the cells need splitting/ passaging.

Protocol for Passaging and Seeding human iPSCs

1. Aspirate the medium from the hiPSC culture.
2. Wash once with 1 mL of 0.5 mM EDTA (in PBS).
3. Aspirate the EDTA and add 1 mL of 0.5 mM EDTA (in PBS) per well of a 6-well plate and incubate the cells for 3 min in a 37°C incubator.
4. Observe the cells under a microscope until the cells at the edge of the colonies start to separate and round up.
5. With the cells still attached, aspirate the EDTA and add 1 mL of mTeSR-plus medium.
6. 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.

7. Transfer the desired dilution to the wells of the new Matrigel®-coated plate (usually around a 1:3 splitting ratio).

   Note: Prepare Matrigel plates (or any other basement matrix) not more than a week prior to passaging cells.

8. Place the plate in the incubator and move the plate back and forth and side to side, twice to spread the clumps evenly in all the wells.

   Note: Human iPSCs are passaged as clumps of 50-200 cells, rather than single cells. Cells need to be passaged before the colonies are large enough to merge with one another.

Protocol for Freezing human iPSCs

1. Label the cryovial as needed, based on 1 vial per well of a 6-well plate, and pre-chill them in a 4°C refrigerator.
2. Aspirate the medium from the hiPSC culture.
3. Wash once with 1 mL of PBS.
4. Aspirate the PBS and add 1 mL of 0.5 mM EDTA (in PBS) per well of a 6-well plate and incubate the cells for 3-5 minutes in a 37°C incubator.
5. Observe the cells under a microscope. After 1-2 minutes the cells at the edge of the colonies will start to separate and round up.
6. Once round colonies are observed, aspirate the EDTA (even if it hasn't been 5 minutes of incubation).
7. Add 1 mL of cold freezing medium, CS10 and scrape the cells from the bottom of the well until the colonies are floating.

   Note: The freezing medium must remain at 4°C until usage.

8. Pipette the cells once or twice to break up any big clumps before transferring the suspension to a cryovial.
9. Transfer the cell suspension into the pre-chilled and labeled cryovial.
10. Place the cryovial in a CoolCell® Freezing Container or in a Styrofoam rack at -80°C overnight, and transfer to liquid nitrogen the next day.

Data

Pluripotency Marker Analysis

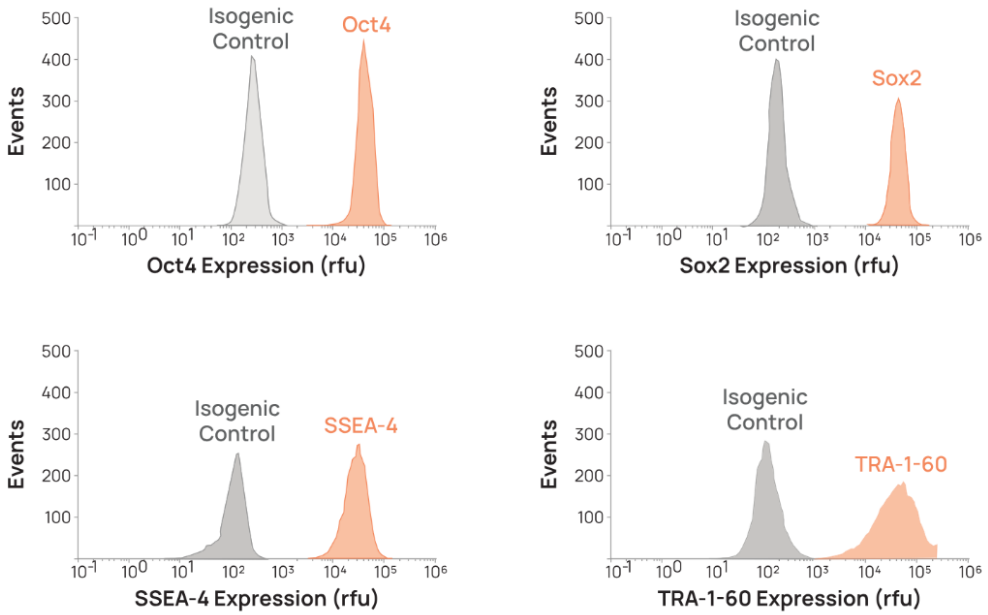

Figure 1. Expression of pluripotency markers. AST-9750 iPS cell line expresses common iPSC biomarkers (top row: OCT-4, SOX2, SSEA-4, TRA-1-60). Bottom row: Corresponding DAPI nuclear staining. All images were taken at 10x magnification.

Karyotype Analysis

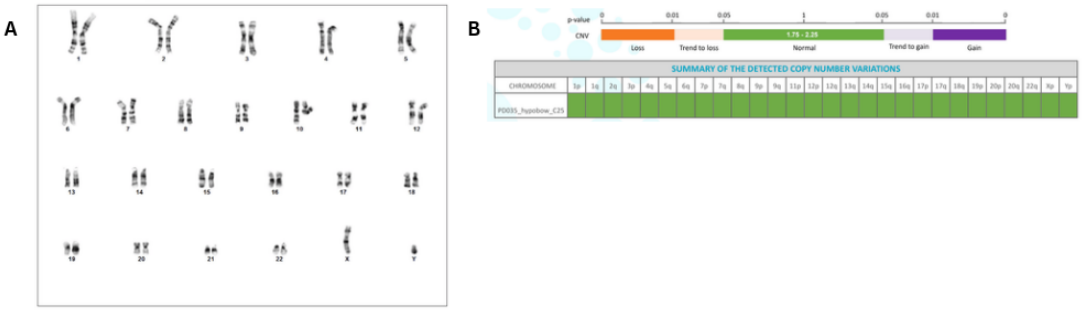

Figure 3. Karyotype analysis to rule out genetic aberrations. This iPSC line demonstrates a normal male karyotype. Cytogenic analysis was performed on twenty G-banded metaphase cells from human iPSC line.

Directed Differentiation into Germ Layers

Genomic stability assessment of AST-9750. Genomic integrity was evaluated using the iCS-digital™ PSC test. No recurrent copy number variations (CNVs) were detected, indicating genomic stability of the cell line.

Related Products

Catalog #	Product	Size
AST-9450	TARGATT™ RUO hiPSC Knock-in Kit	1 Kit

Technical Support

CONTACT US

For more information or assistance, contact Customer Service at:

- Email: [info@appliedstemcell.com]
- Direct line: [(408) 773-8007]

WEBSITE RESOURCES

Visit appliedstemcell.com for technical resources and information including:

- Safety Data Sheets (SDS)
- Certificates of Analysis (when available)
- FAQs
- Product literature
- Complete contact information

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