Applied StemCell

BIOLOGICAL RELEVANCE.
ENGINEERED PRECISION.
GMP EXPERTISE.

ActiCells TARGATT™ RUO Hypo hiPSC Knock-in Kit

Reprogrammed from CD34+ cord blood cells and rendered hypoimmunogenic by knocking-out both the β2 microglobulin (B2M) gene and the HLA class II transactivator (CIITA) gene, the TARGATT™ RUO Hypo hiPSC Knock-in Kit (Cat. #AST-9650) is a fast and reliable way to build your allogeneic cell-based medicine.

Designed to jumpstart your allogeneic development, the TARGATT™ RUO Hypo hiPSC Knock-in Kit gives you a hypoimmunogenic cell line that is immediately ready for further modification. Knock-in additional immune evasion elements to avoid clearance by NK cells, macrophages, and dendrites while also expressing disease-modifying genes. The power of the TARGATT™ platform means you can insert all these genes in a single reaction—up to 20 kb—with additional DNA over 20 kb added in nested reactions.

Parental Line

Control human iPSCs (ASE-9280)
Gender: Male
Tissue Source: CD34+ Umbilical Cord Blood Cells
Reprogramming Method: Episomal

Catalog #	Product	Size
AST-9650	ActiCells RUO Hypo hiPSC Knock-in Kit	1 kit

Intended Use

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

Quality Assurance

A certificate of analysis (CoA) with detailed quality control information for the cell line and components of the kit will be provided with each shipment.

Storage Instructions and Stability

Store the TARGATT™ master cell line in liquid nitrogen freezer immediately upon receipt. Store the plasmids at -20°C. Do not freeze-thaw plasmids repeatedly. The products provided in this kit are stable for at least 6 months from the date of receiving when stored as directed.

Precautions

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.

Directions for Use

Kit Components

Catalog #	Component	Amount
AST-9650-C	TARGATT™ RUO hypo hiPSC Line	1 x 10^6 cells
AST-3091	TARGATT™ 46 CAG-MCS Cloning Plasmid	5 µg
AST-3096	TARGATT™ 56 CAG-GFP Positive Control Plasmid	15 µg
AST-3084	TARGATT™ CAG-integrase Plasmid	15 µg

1. Thawing and culturing cryopreserved cells

To ensure the highest level of viability, thaw the vial containing the cells and culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1.1 Prepare 4 mL of mTeSR™ Plus media (containing 1X Pen/Strep) with 10 µM Rock Inhibitor (mTeSR™ Plus media + Rock Inhibitor).
1.2 Add 1 mL of mTeSR™ Plus media + Rock Inhibitor in one well of a Matrigel® coated 6-well plate. Prepare 2 wells for each vial of frozen cells.
1.3 Use dry ice to bring one vial of frozen AST-9650c cells to the cell culture room. Quickly thaw it in a 37°C water bath by gently shaking the cryovial continuously until only a small piece of ice remains.
1.4 Wipe the cryovial with a paper towel sprayed with 70% ethanol and place in a biosafety hood.
1.5 Add 9 mL of mTeSR™ Plus in a 15 mL-conical tube.
1.6 Use a 5 mL serological pipette to transfer the cells to the 15 mL tube.
1.7 Centrifuge the cells at 200 x g for 5 minutes at 4°C.
1.8 Aspirate the medium and add 2 mL of mTeSR™ Plus media + Rock Inhibitor.
1.9 Gently flick the conical tube to resuspend the cells and transfer them to the 2 wells of the Matrigel® coated plate.
1.10 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.

2. Sub-culturing procedure (culture maintenance)

2.1. The next day, aspirate the medium and add add 2 mL of fresh mTeSR™ Plus into each well.
2.2. Change the medium every day. For medium change, aspirate off the spent medium and add 2 mL of fresh mTeSR-plus medium in each well.
2.3. When the colonies are big enough or close to merging, the cells need splitting

Note: To ensure the best quality of cells, the cell culture should be passaged every 4-6 days

2.4. Aspirate the medium from the culture wells
2.5. Wash once with 1 mL of 0.5 mM EDTA (in PBS).

2.6. Aspirate the PBS, add 1 mL of 0.5 mM EDTA per well and incubate the cells for 3 minutes in a 37°C incubator.
2.7. Observe the cells under the microscope. The cells at the edge of the colonies will start to separate and round up.
2.8. With the cells still attached, aspirate the EDTA and add 1 mL of mTeSR™ Plus medium.
2.9. Scrape the cells from the bottom of the well until all the colonies are floating; pipette up and down 2-3 times to break the colonies into small clumps.
2.10. Make a 1:10 dilution and transfer the cells to the wells of the new Matrigel® coated plates.
2.11. Place the plate in the CO_2 incubator and move the plate back-and-forth and side-to-side twice to spread the clumps evenly in the wells.

3. Cryopreserving cells

3.1. Label the cryovials as needed, based on 2 vials per well of a 6-well plate, and pre-chill them in a 4°C freezer.
3.2. Aspirate the medium from the hiPSC culture.
3.3. Wash once with 1 mL of 0.5 mM EDTA (in PBS).
3.4. Aspirate the PBS and add 1 mL of 0.5 mM EDTA per well in 6-well dish and incubate the cells for 3 minutes in a 37°C incubator.
3.5. Observe the cells under microscope until the cells at the edge of the colonies start to separate and round up.
3.6. With the cells still attached, aspirate the EDTA and add 2 mL of CryoStor® CS10 medium.
3.7. 3Scrape the cells from the bottom of the well until the colonies are all floating.
3.8. Aliquot the cell suspension in 2 pre-chilled and labeled cryovials: 1 ml in each vial.
3.9. 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.

4. Transfection procedure

4.1. Plasmid amplification and cloning with TARGATT™ 46 CAG-MCS cloning plasmid

4.1.1 Amplify the plasmids using NEB® 10-beta Competent E. coli (High Efficiency) competent cells

Note: Do not use 5-alpha competent cells to transform the plasmids

4.1.2 Clone the gene of interest into the TARGATT™ 46 CAG-MCS Cloning Plasmid to make the donor plasmid.

4.1.3 Aliquot into single-use quantity and store at -20°C. Do not freeze-thaw the plasmid repeatedly.

Note: We recommend using 5 µg of the plasmid for each transfection

4.2 Transfection using Lonza® 4D-nucleofector transfection system

4.2.1 Culture the TARGATT™ hiPSCs until the cells reach a 50% confluency.
4.2.2 Replace the complete medium with warm medium without Pen/Strep medium and culture overnight.
4.2.3 Aspirate the medium.
4.2.4 Wash the cells once with 1 mL PBS.
4.2.5 Add 1 mL TrypLE into each well and incubate for 5 minutes at 37°C.
4.2.6 Mix the cells by pipetting about 5 times and transfer the cells into a 15 mL conical tube.
4.2.7 Add 5 mL of mTeSR™ Plus medium to neutralize the enzyme.
4.2.8 Centrifuge at 200 x g for 5 minutes at 4°C.
4.2.9 Aspirate the supernatant and resuspend the cells in 1 mL PBS and count cell density.
4.2.10 Transfer 1 million cells into a 1.5 mL Eppendorf tube® and centrifuge at 200 x g for 5 minutes at 4°C.
4.2.11 Aspirate the supernatant and resuspend the cells in 100 µL transfection mixture (Table 1) containing the gene of interest cloned into the TARGATT™ 46 plasmid (donor plasmid) and 1 µg of integrase plasmid (AST-3084) and Lonza buffer.

Table 1. Preparation of the transfection mixture

Reagent	Amount
Donor plasmid containing transgene	4 µg
TARGATT™ CAG-integrase plasmid (AST-3084)	1 µg
P3 Primary Cell NucleofectorTM Solution	82 µL
Supplement 1	18 µL
Final volume	**100 µL**

4.2.12 Load the cell-DNA mixture into NucleocuvetteTM Vessel and electroporate the cells using the Lonza™ electroporation system and the following conditions: CA-137.
Note: If using other transfection methods, please optimize your protocol accordingly.

4.2.13 After electroporation, transfer the cells into 3 wells of a Matrigel® coated 6-well plate, culture in non- Pen/Strep mTeSR™ Plus medium + Clone R for 24 hours.

4.2.14 After 24 hours, the medium is refreshed with complete mTeSR™ Plus medium.

4.2.15 The cells will recover in 3-4 days.

4.3 Enrichment protocol

Selection strategy using Blasticidin:
The Blasticidin treatment (positive selection) can remove the non-transfected cells as well as enrich for cells with transgene integrated into the H11 locus.

4.3.1 Three (3) days after electroporation, expand the cells 20-fold. Use Tryple- Select to digest the cells as single cells.

4.3.2 Plate the single cells in new plate and after 24-hours of culture, replace the growth medium with Blasticidin selection media. (growth media + Blasticidin (10 ug/mL)).

4.3.3 Maintain the selection pressure for 2 weeks, or until the desired level of selection is achieved (can be monitored by checking for mCherry fluorescence using microscope.

4.3.4 Expand the cells for further downstream processing.

4.4 Single cell cloning

4.4.1 On day 12 post-electroporation, dissociate the cells using 1 mL Trypsin LE for 5 minutes into single cells (following step 4.2.5).

4.4.2 Seed the single cell iPSCs into a Matrigel® coated 96-well plate with 80 µL of mTeSR™ Plus medium + Rock Inhibitor and sort the cells using BD-Melody FACS machine.
Note 1: If you are using a FACS machine by a different manufacturer, please optimize your sorting protocol.
Note 2: If you do not have a FACS instrument or do not want to use FACS for single cell sorting, you could also do a serial dilution.

4.4.3 When the single cell clones grow into 4-8 cell clusters in about 4-5 days, replace the spent medium with 100 µL fresh mTeSR™ Plus medium.

4.4.4 Change the medium every 2 days. For medium change, aspirate off the spent medium and add 100 µL of fresh mTeSR-plus medium in each well.

4.4.5 When the cells reach 10% confluency, transfer each well of cells from the 96-well plate into one-well of a fresh Matrigel® coated 48-well plate.

4.4.6 When the cells reach 60% confluency, passage 50% of the cells into fresh Matrigel® coated 6-well plates and use the other 50% of the cells for genotyping.

Data

Figure 1. [Title] [Description]

Figure 2. Knocking out cell surface expression of HLA I and HLA II eliminates the T cell response, although other gene edits are needed to address innate immune responses. A homozygous double knock-out of the B2M and CIITA genes was created in a hiPSC line containing a TARGATT™ landing pad.

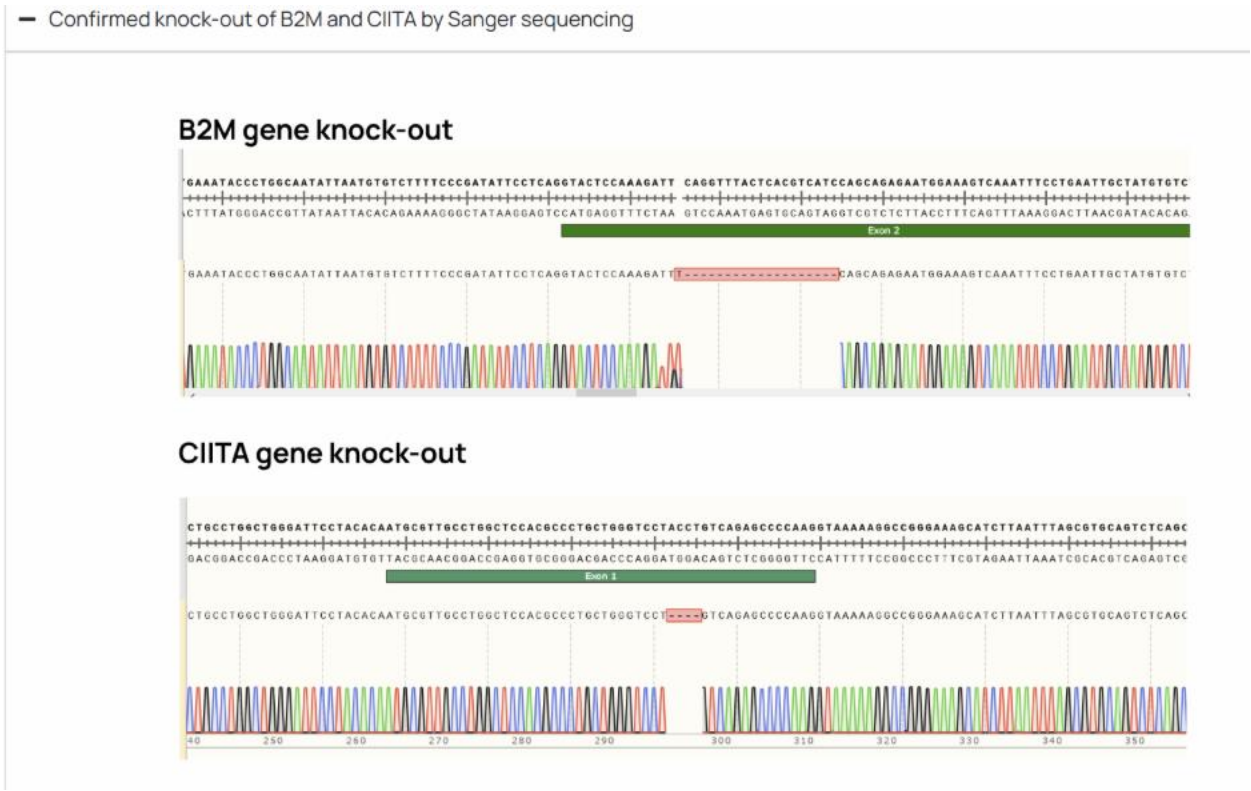

Figure 3. Sanger sequencing confirms homozygous knock-out at both the B2M and CIITA loci.

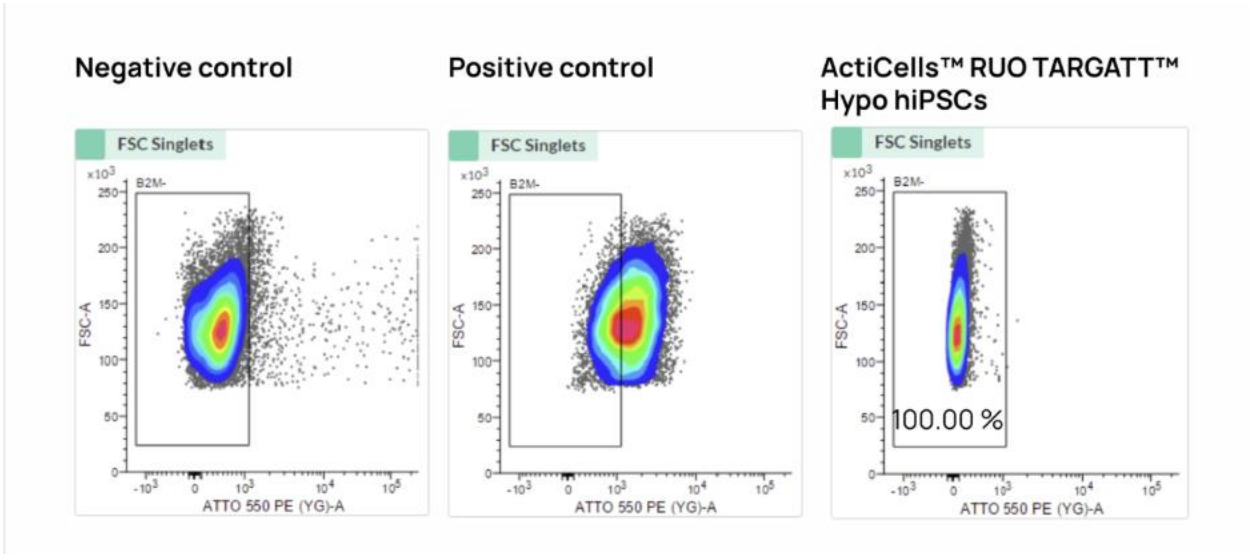

Figure 4. Loss of B2M expression confirmed using an anti-B2M antibody and flow cytometry.

Figure 5. Pluripotency confirmed by flow cytometry using the relevant antibodies.

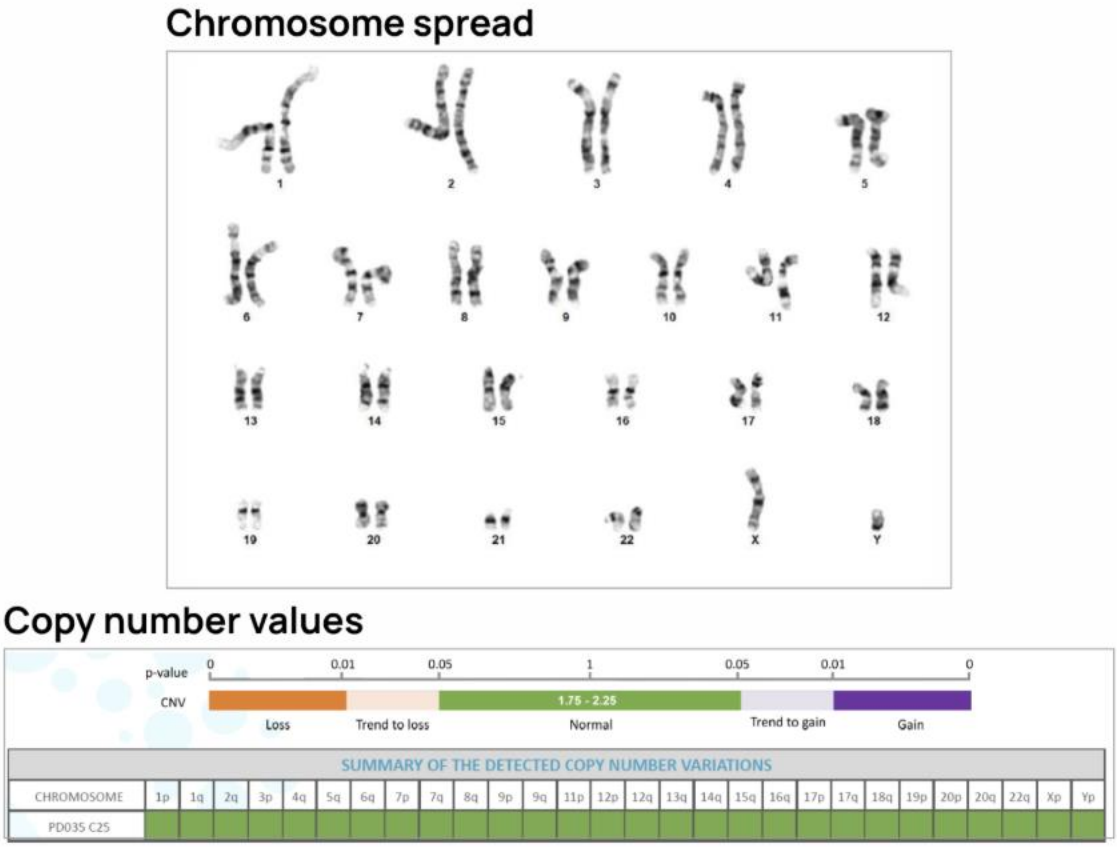

Figure 6. Normal karyotype confirmed for the parental line.

Related Products

Catalog #	Product	Size
AST-9450	ActiCells GMP-Matching RUO TARGATT 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

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Applied StemCell, Inc.
521 Cottonwood Dr, Suite # 111, Milpitas, California, 95035, USA • Telephone: +1 866.497.4180 • appliedstemcell.com