Description

The B2M Knockout iPS Cell Line is an iPS cell line that has been edited using CRISPR/Cas9 technology to delete B2M (Beta-2-Microglobulin) gene expression. The B2M-targeting CRISPR/Cas9 editing agents were delivered via transduction with B2M (Human) CRISPR/Cas9 Lentivirus (Non-Integrating) (BPS Bioscience #78341).

Background

The discovery by Yamanaka and colleagues in 2007 that 4 factors were sufficient to reprogram terminally differentiated fibroblasts into pluripotent stem cells launched the advent of human induced pluripotent stem (iPS) cell technology. These human iPS cells are capable of both self-renewal and differentiation down all three germline lineages and provide both a tool to model human development and disease in the relevant differentiated human cell types, and a unique opportunity for high throughput drug screening and cell therapy development.

B2M (Beta-2-Microglobulin) forms a heteromeric complex with HLA-A, HLA-B and HLA-C molecules to generate the functional Class I MHC molecule responsible for antigen presentation to T-Cells. Class I MHC molecules are present on the surface of all nucleated cells and play a role in the rejection of organs or allogenic cells during organ transplantation and cell therapy. B2M is an attractive target to reduce the immunogenicity of iPS cell-derived allogenic cell therapies.

Application(s)

- Assess the role of B2M knockout in iPS cell biology and differentiation.
- Assess the impact of B2M knockout in cell therapy research and development.

Considerations

Maintenance of the cells requires specific reagents such as specialty culture media, Matrigel™, Accutase™, RelesR™, and Thiazovivin that are not provided with the cells. Ensure that you have all reagents on hand prior to thawing the cells. Prepare media as indicated in section *"Media Required for Cell Culture"* below.

Thiazovivin is a Rho Kinase inhibitor used to ensure that sensitive cell types such as iPS cells survive cell dissociation process and re-plate successfully. Thiazovivin is not stable in solution and should be added to the medium immediately before use.

Materials Provided

Components	Format
1 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of iPS Cell Freezing Medium

Parental Cell Line

Non-Disease Human iPS Cell Line (iXCells 30HU-002)

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
mTeSR™ Plus	Stem Cell Technologies #100-0276
Matrigel™	Corning #354230
DMEM/F12	Thermo Fisher #11330032
Thiazovivin	BPS Bioscience #78506
RelesR	Stem Cell Technologies #05872
Accutase	Thermo Fisher #A1110501

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage.
Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media formulations by BPS Bioscience is *highly recommended*. Other formulations of media may result in suboptimal performance.

Note: Cells should be cultured at 37℃ with 5% CO_2.

Media Required for Cell Culture

iPSC Thaw Medium:
mTeSR™ Plus supplemented with 1% Penicillin/Streptomycin.

Complete iPSC Thaw Medium:
mTeSR™ Plus supplemented with 1% Penicillin/Streptomycin, and 1 µM Thiazovivin.

Thiazovivin is unstable once diluted in the medium. Prepare just enough to proceed with thawing of the cells.

iPSC Growth Medium:
mTeSR™ Plus supplemented with 1% Penicillin/Streptomycin.

PSC Passage Medium:
mTeSR™ Plus supplemented with 1% Penicillin/Streptomycin, and 1 µM Thiazovivin.

2X IPS Cell Freezing Medium:
80% mTeSR™ Plus supplemented with 1% Penicillin/Streptomycin, 1 µM Thiazovivin, and 20% DMSO (vol/vol).

Cell Culture Protocols

Note: iPS cells are derived from human material and thus the use of adequate safety precautions is recommended.

Matrigel®-coated plate preparation

Matrigel™ solidifies rapidly when warm. Keep everything on ice and work in sterile conditions. Matrigel™ coated plates can be prepared up to two weeks ahead of time. We recommend following the manufacturer's instructions for Matrigel™ handling. Matrigel™ should not be subjected to repeated freeze-thaw cycles. When first using a vial of Matrigel™, it is recommended to aliquot ~100 µl and/or ~200 µl into microcentrifuge tubes for future use.

1. Prepare cold, sterile cell culture medium such as DMEM/F12 containing 1% Penicillin/Streptomycin (no serum).

2. Thaw Matrigel™ at 4°C.

3. While the Matrigel™ is thawing, transfer the desired volume of ice-cold DMEM/F12 into a 50 ml conical tube.

Table 1: Example of volumes to be used with various size plates or flasks.

Cell culture plate	Matrigel™ volume	Volume medium	Coating volume
2x 6-well plate	~100 µl*	25 ml	2 ml/Well
4x 6-well plate	~200 µl*	50 ml	2 ml/Well
4x 96-well plate	~100 µl*	40 ml	100 µl/Well
4x T25 Flask	~100 µl*	12 ml	3 ml/Flask
3x T75	~200 µl*	30 ml	10 ml/Flask
2x T175	~ 300 µl*	40 ml	20 ml/Flask

** Amount is lot-specific, please refer to manufacturer's CoA.*

4. Once Matrigel™ is thawed, add 500 µl of cold DMEM/F12 to the microcentrifuge tube containing the Matrigel.

5. Pipette up and down using a 1 ml pipette tip.

6. Transfer the diluted Matrigel™ aliquot to the 50 ml conical tube containing the ice-cold medium.

7. Plate the Matrigel™ solution in the cell culture plates according to coating volumes shown in Table 1.

8. Transfer to a CO_2 Incubator at 37°C for a minimum of 1 hour and up to 2 weeks.

Note: The DMEM/F12 medium must be gently removed from the Matrigel™-coated wells immediately before adding the cells.

Cell Thawing

1. Ensure that you have prepared the Matrigel™-coated culture plates or flasks at least 1 hour in advance.

2. Bring iPSC Thaw Medium to Room Temperature (RT). **iPSC Thaw Medium should NOT be pre-warmed in a water bath.**

3. Prepare 15 ml of Complete iPSC Thaw Medium by adding Thiazovivin to a final concentration of 1 µM.

Thiazovivin is unstable once diluted in the medium. Prepare just enough to proceed with thawing the cells.

4. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

5. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to an empty 50 ml conical tube.
   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

6. Using a 10 ml serological pipette, slowly add 10 ml of Complete iPSC Thaw Medium to the conical tube containing the cells. iPSC Thaw Medium should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

7. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and gently resuspend the cells in 5 ml of Complete iPSC Thaw Medium.

8. Aspirate coating solution from 2 wells of a 6-well Matrigel™-coated plate.

9. Immediately transfer the resuspended cells to the 2 wells of the Matrigel™-coated plate and incubate at 37°C in a 5% CO_2 incubator. Each well contains approximately 1 million cells.

10. Rock the plate to ensure uniform distribution of cells.

11. After 24 hours in culture, check for cell attachment and viability. Change the culture medium to fresh iPSC Thaw Medium and continue growing cells in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

12. Cells should be passaged before they reach 80% confluency or before colonies become too large, whichever comes first. For the first passage pass cells in complete iPS Cell Thaw Medium (including 1 µM thiazovivin), and switch to iPS Cell Growth Medium the following day. For all subsequent passages, use iPS Cell Growth Medium, following the passage protocol below.

13. Perform media changes as recommended in the cell maintenance schedule below. For B2M Knockout iPS Cells cultivated in mTeSR Plus, we recommend one media change on either Saturday or Sunday. This is a recommended schedule only, cells should be fed and passaged based on daily visual observation.

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Passage	Change medium	No Change	Passage	Change medium	One medium change	

Routine Cell Passage

1. Monitor iPS Cell cultures for both colony size and plate confluence. Passage once the colonies are large with a dense, tightly packed central region or when the well is ~80% confluent, whichever occurs first.

2. Ensure that you have prepared Matrigel™-coated culture plates or flasks at least 1 hour in advance.

3. Prepare 15 ml of Passage Medium by adding Thiazovivin to a final concentration of 1 µM to 15 ml of Growth Medium.

4. Aspirate spent cell culture medium, and gently wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}.

5. Add 1 ml of RelesR™ per well of a 6-well plate and rock the plate to evenly distribute RelesR™. Immediately aspirate all but ~60 µl of RelesR™ leaving a very thin film.

6. Incubate at 37°C for 3-5 minutes or until the edges of the iPS Cell colonies have begun to detach (the colonies will appear to be "curling up" from the edges).

7. Once the cells have detached, add iPSC Passage Medium, transfer to a tube and dilute with Passage Medium to seed into new Matrigel-coated culture vessels at a sub-cultivation ratio of 1:10 to 1:20. Be sure to aspirate the coating solution from Matrigel-coated plates before plating the cells.

Cell Freezing

1. Add Thiazovivin to make a final concentration of 1 µM to 15 ml of iPSC Thaw Medium to make Complete iPSC Thaw Medium.

2. Prepare 2X Freezing Medium: 80% Complete iPSC Thaw Medium + 20% DMSO.

3. Aspirate the cell culture medium and wash the cells with PBS without Ca^{2+}/Mg^{2+}.

4. Add 1 ml of RelesR™ per well of a 6-well plate and rock the plate to evenly distribute RelesR™. Immediately aspirate all but ~60 µl of RelesR™ in order to leave a very thin film of liquid covering the cells.

5. Incubate at 37°C for 3-5 minutes or until the edges of the iPS Cell colonies have begun to detach (the colonies will appear to be "curling up" from the edges).

6. Once the cells have detached, add Complete iPSC Thaw Medium and count the cells. For routine use, two vials can be frozen from a ~80% confluent well of a 6-well plate. Alternatively, cells can be frozen at 2 million cells/vial.

7. Spin down the cells at 300 *x g* for 5 minutes, remove the supernatant and resuspend the cells in Complete iPSC Thaw Medium using 0.5 ml of medium per vial to be frozen.

8. Using a 10 ml serological pipette, slowly add an equal volume of 2X Freezing Medium (0.5 ml per vial to be frozen) to the conical tube containing the iPS Cells. The 2x Freezing Medium should be added dropwise while softly rocking the conical tube to permit gentle mixing and avoid osmotic shock.

9. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

10. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

A. Pluripotency marker expression in B2M Knockout iPS Cell Line

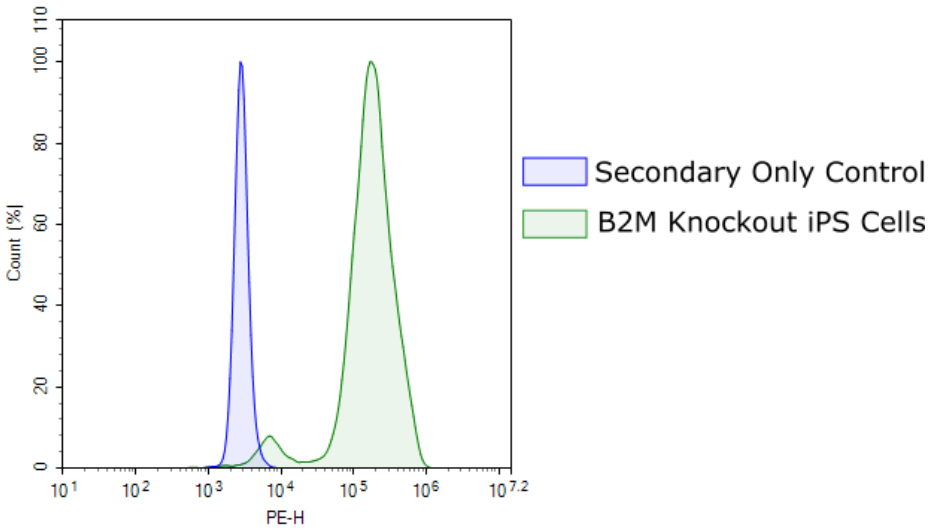

Figure 1. Flow cytometry analysis of OCT expression in B2M Knockout iPS Cell Line.
Cells were fixed with Fixation Buffer (BioLegend #42080) and intracellular staining was performed with an anti-Oct4 antibody (BioLegend #653701) followed by a PE goat anti-mouse IgG secondary antibody (BioLegend #405307). B2M Knockout iPS Cell Line expression of OCT4 (green) was compared to cells stained with secondary antibody only as control (blue).

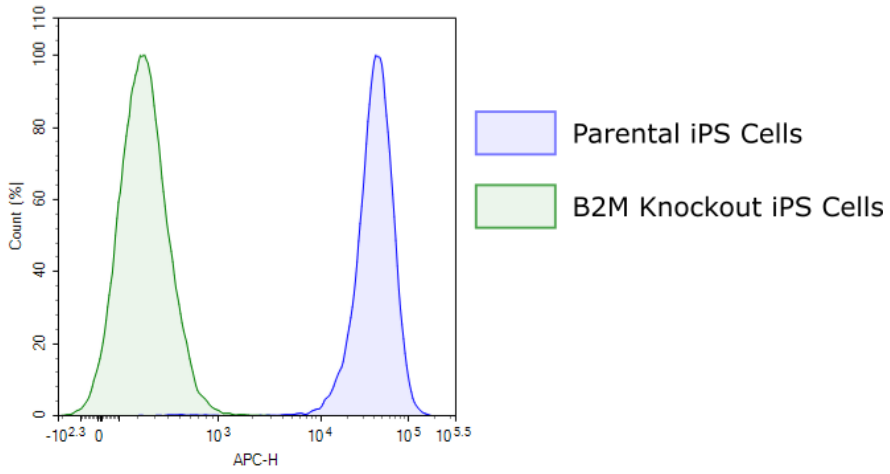

Figure 2: Flow cytometry analysis of B2M expression B2M Knockout iPS Cell Line.
Cell surface staining was performed with an APC anti-human β2 antibody (Biolegend #316312) on the B2M Knockout iPS Cell Line (green) and parental iPS Cell control cells (blue) to confirm lack of expression in the B2M Knockout iPS Cell Line.

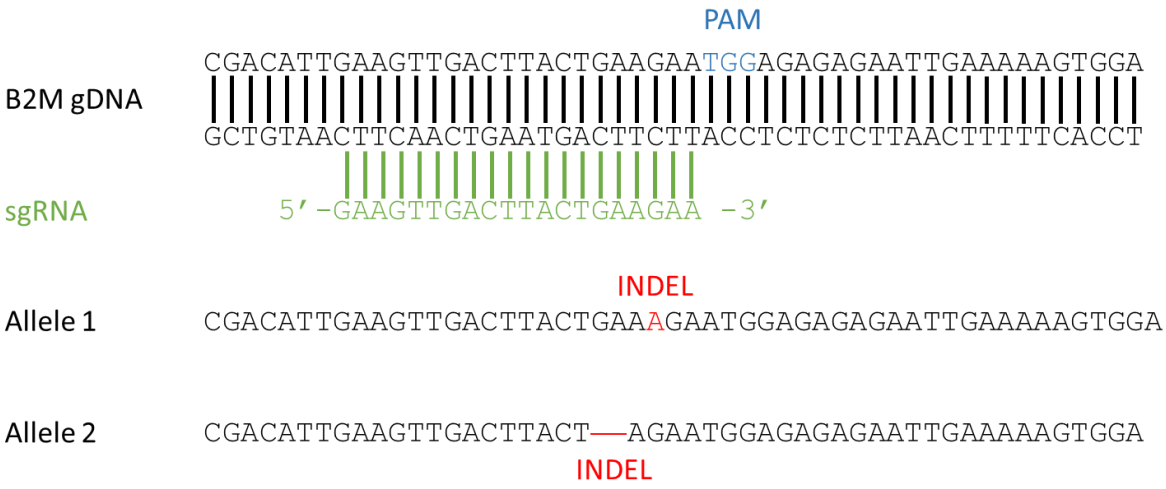

Figure 3: Genome sequencing of B2M in the B2M Knockout iPS Cell Line.
Genomic DNA was extracted from the B2M Knockout iPS Cell line and submitted for sequencing. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) in green and the Indels (Insertions/Deletions) in the two B2M alleles are shown in red.

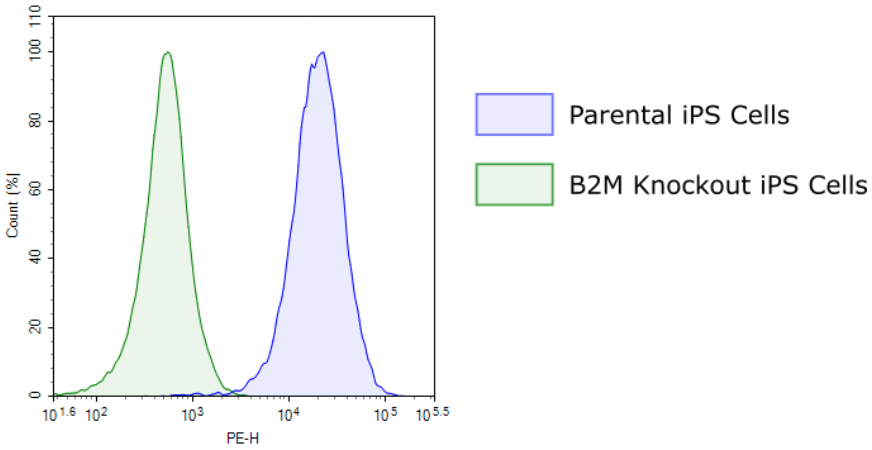

Figure 4: Flow cytometry analysis of HLA expression in B2M Knockout iPS Cell Line.
Cell surface staining was performed with a PE anti-human HLA-A, B, C Antibody (Biolegend #311405) on the B2M Knockout iPS Cell Line (green) and parental iPS Cell control cells (blue) to confirm lack of cell surface HLA expression in the B2M Knockout iPS Cell Line.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

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License Disclosure

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Related Products

Products	*Catalog #*	*Size*
Cas9 Expressing iPS Cell Pool	78578	1 vial
Cas9 Inducible (Tet-On) iPS Cell Pool	78845	1 vial
B2M (Human) CRISPR/Cas9 Lentivirus (Integrating)	78340	500 µl x 2
B2M (Human) CRISPR/Cas9 Lentivirus (Non-Integrating)	78341	500 µl x 2

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