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- Trockeneiszuschlag
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Description

The Human B2M Knockout iPSC-Derived Cardiomyocytes are non-diseased, non-proliferative human cardiomyocytes differentiated from B2M Knockout iPS Cell Line (#82161). The iPS cell line was generated using CRISPR/Cas9 technology to remove B2M (Beta-2-Microglobulin) gene expression. The B2M-targeting CRISPR/Cas9 editing reagents were delivered via transduction with B2M (Human) CRISPR/Cas9 Lentivirus (Non-Integrating) (#78341).

Background

The discovery of the Yamanaka factors has enabled the reprogramming of mature human somatic cells to induced pluripotent stem cells (iPSCs) with the ability to differentiate along the three germlines lineages involved in human development (endo-, meso- and ectoderm). The impact of this discovery has been most profound in research involving terminally differentiated, non-proliferating cell types which have traditionally been difficult to access.

One of the major causes of the death and burden on the health systems in the developed world are cardiovascular diseases and human iPSC-derived cardiomyocytes have furthered our understanding of human cardiac development, congenital heart diseases and mechanisms of drug-induced cardiotoxicity. Importantly, human iPSC derived cardiomyocytes also serve as a source for cell therapies to repair damaged heart tissue. Several strategies are currently under clinical trial for cardiomyocyte cell-replacement therapies, including the transplantation of dissociated cells, engineered heart tissues patches and cardiac spheroids. The goal of any cardiac cell therapy is engraftment of the *in vitro*-generated cardiomyocytes onto the recipient's heart and integration of structural and electrophysiological systems to support recipient cardiac function. Similar to whole-organ transplantation, iPSC derived cell therapies are subject to mechanisms of immune rejection, which can decrease the efficiency of cell engraftment and retention which impedes the therapeutic effect. 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 one of several attractive targets to reduce the immunogenicity of iPS cell-derived allogenic cell therapies. While the current cardiomyocyte cell therapies undergoing clinical trial do not leverage hypoimmune edits to provide immune cloaking, several are under development in preclinical pipelines.

Application(s)

- Assess the impact of B2M knockout on iPSC derived cardiomyocyte function.
- 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™, 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 iPSC and iPSC-derived cells survive the 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 5 x 10^6 cells in 1 ml of STEMdiff Cardiomyocyte Freezing Media (Stem Cell Technologies #05030)

Parental Cell Line

PBMC-derived, non-disease Human iPS Cell Line (XCells 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
Thaw Medium Kit C17	BPS Bioscience #78511
Maintenance Medium C17	BPS Bioscience #78509
Matrigel™	Corning #354230
DMEM/F12	Thermo Fisher #11330032
Thiazovivin	BPS Bioscience #78506

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 from BPS Bioscience is highly recommended. Other preparations or formulations of media may result in suboptimal performance.

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

Media Required for Cell Culture

Thaw Medium Kit C17 (BPS Bioscience #78511):

Component A: Maintenance Medium C17

Component B: 10% Serum Replacement

Component C: 10 mM Thiazovivin

Thaw Medium C17 Preparation (prepare immediately before use): add 5 ml of Component B and 50 µl of Component C to 45 ml of Component A in a sterile tissue culture hood. Do not filter.

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

Maintenance Medium C17 (BPS Bioscience #78509):
RPMI 1640 medium supplemented with 5% B27 Supplement, 1% Penicillin/Streptomycin.

Do not warm Maintenance Medium C17 in a water bath. Bring to room temperature on the bench or tissue culture hood before use.

Cell Culture Protocols
Note: iPS cells are derived from human material and thus the use of adequate safety precautions is recommended.
Human B2M Knockout iPSC-Derived Cardiomyocytes are non-proliferative and cannot be expanded using standard cell culture methods. BPS Bioscience cryopreserved cardiomyocytes should be thawed and plated directly at the cell density needed for the assay of interest.

Matrigel®-coated plates

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 plate or flask at least 1 hour in advance.

2. Bring Maintenance Medium C17 to Room Temperature (RT).

Note: Maintenance Medium C17 should NOT be pre-warmed in a water bath.

3. Prepare 50 ml of fresh Thaw Medium C17 and mix well.

4. Retrieve a cell vial from liquid nitrogen storage. Keep the vial in 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 Thaw Medium C17 to the conical tube containing the cardiomyocytes. The 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 200 *x g* for 3 minutes.

8. Carefully remove the medium and gently resuspend the cell pellet in 5 ml of Thaw Medium C17.

9. Count the cells.

10. Aspirate coating solution from the Matrigel™-coated plates and seed the cells at the desired cell density.

Note: The desired density depends on the specific application, which may require sparse or confluent cells. As a reference, plating live cells (as determined by a trypan blue staining method) at 50,000 cells/well in a 96-well plate usually results in ~90% confluency.

11. After 24 hours of culture, check for cell attachment and viability. Change the medium to fresh Maintenance Medium C17 and maintain in a 5% CO_2 incubator at 37°C.

12. Change the medium every 2-3 days until the cells are needed for the assay.

    Note: Human B2M Knockout iPSC-Derived Cardiomyocytes are non-proliferative. Cells can be maintained for up to 7 days after plating prior to performing the experiment.

Validation Data

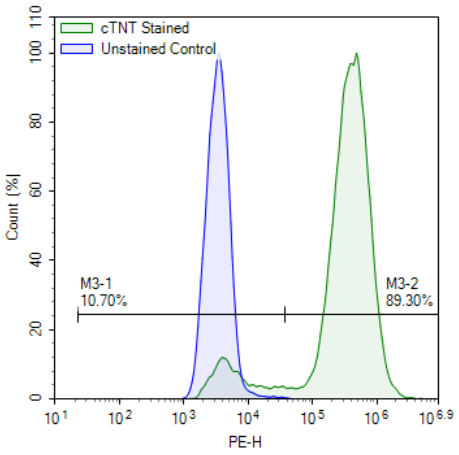

Figure 1. Cardiac Troponin-T (cTNT) Expression in Human B2M Knockout iPSC Derived Cardiomyocytes by flow cytometry.
Cells were fixed with Fixation Buffer (BioLegend #42080) and intracellular staining was performed with PE Mouse Anti-Cardiac Troponin T antibody (BD #653701). Human B2M Knockout iPSC Derived Cardiomyocytes expression of cTNT (green) was compared to unstained cells as control (blue). The y axis represents the % of cells, while the x axis indicates the PE intensity.

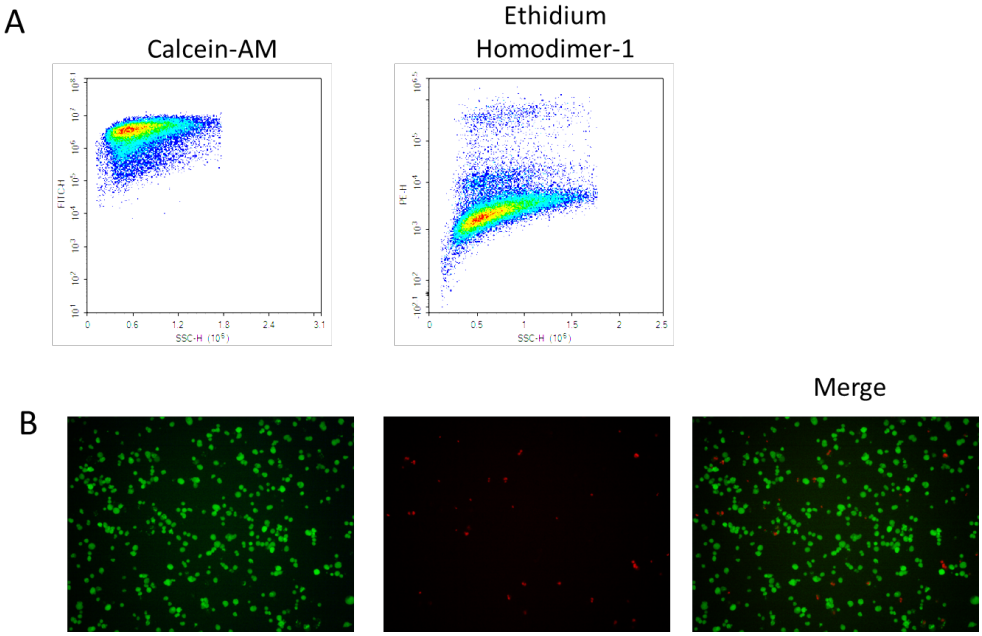

Figure 2. Cell viability and plating efficiency of Human B2M Knockout iPSC Derived Cardiomyocytes at thaw.

Human B2M Knockout iPSC Derived cardiomyocytes were thawed as described in the above protocol. Cell viability was measured using Live/Dead Viability/Cytotoxicity Kit for mammalian cells (ThermoFisher #L3224) and assessed by **A.** flow cytometry and **B.** fluorescence microscopy. Calcein AM (green) staining indicates live cells, while Ethidium Homodimer-1 (red) stains dead cells.

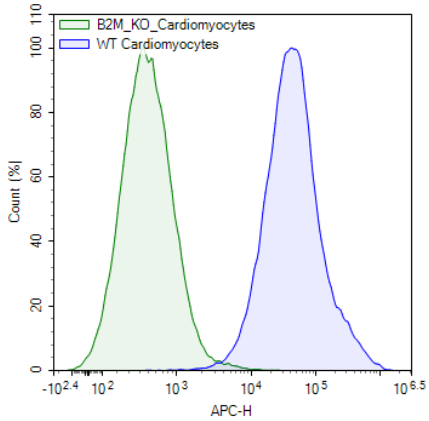

Figure 3: B2M expression in Human B2M Knockout iPSC Derived Cardiomyocytes analyzed by flow cytometry.

Cell surface staining was performed with an APC anti-human β2-microglobulin Antibody (BioLegend #316312) on the Human B2M Knockout iPSC Derived Cardiomyocytes (green) and WT Human iPSC Derived Cardiomyocytes (#78529) (blue) to confirm lack of expression in B2M Knockout iPSC Derived Cardiomyocytes.

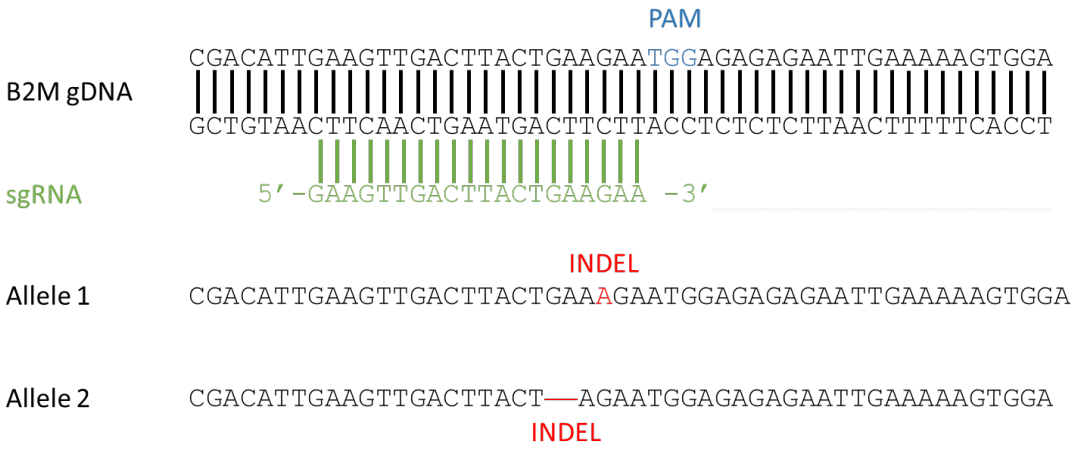

Figure 4: Genome sequencing of B2M in the undifferentiated B2M Knockout iPS Cell Line (#82161).
Genomic DNA was extracted from the B2M Knockout iPS Cell Line (#82161) and submitted for sequencing. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) in green and the Indels (Insertions/Deletions) in the two B2M alleles are shown in red.

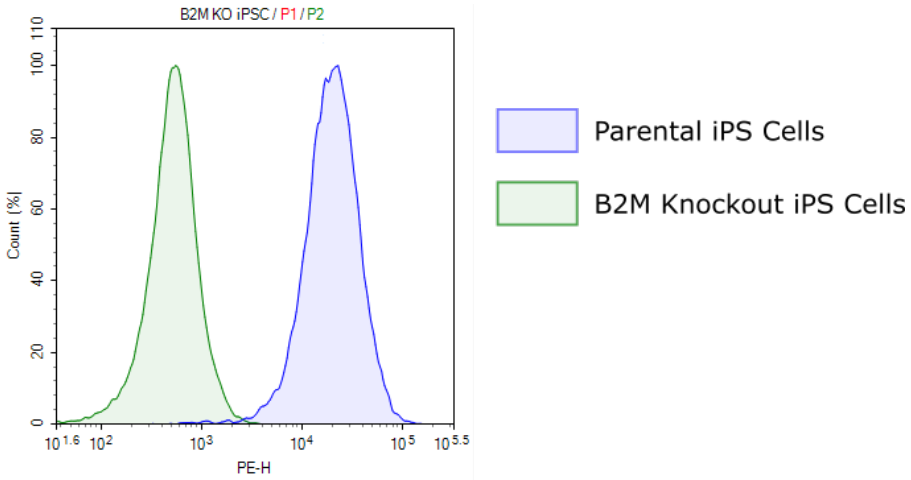

Figure 4: HLA expression in Human B2M Knockout iPS Cell Line analyzed by flow cytometry. Cell surface staining was performed with a PE anti-human HLA-A,B,C Antibody (BioLegend #311405) in B2M Knockout iPS Cell Line (green) (#81161) and parental iPS cells, to confirm lack of cell surface HLA expression in 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

The iPSC technology is protected by several patents, including US patent Nos. 8048999, 8058065, 8129187, 8278104, 8530238, 8900871, 9404124, 9499797, 10519425, and patent pending, for which iPS Academia Japan, Inc. has been granted license rights with a sub-licensable right.

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Related Products

Products	*Catalog #*	*Size*
B2M Knockout iPS Cell Line	82161	1 vial
Human iPSC Derived Cardiomyocytes	78529	Various sizes
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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