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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

Human iPSC Derived Cardiomyocytes are non-diseased, non-proliferative human cardiomyocytes differentiated from induced pluripotent stem cells (iPSC) using the small molecule Wnt-modulation strategy described by Lian *et al.*. The differentiated cells are functional, normal cardiomyocytes useful for *in vitro* modeling of cardiac biology and drug development studies.

Background

The discovery of the Yamanaka factors has enabled the reprogramming of mature human somatic cells to induced pluripotent stem cells 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. Human iPSC-derived cardiomyocytes have enhanced our understanding of human cardiac development, congenital heart diseases and mechanisms of drug-induced cardiotoxicity. In addition, the availability of human cardiac muscle cells can transform the drug discovery process. On the one hand, it allows high-throughput phenotypic screening of new drugs targeting cardiac disease. On the other hand, it allows cardiotoxicity studies to be performed very early on in the drug discovery without using more expensive, and ultimately less clinically relevant mouse models. These two aspects combined are expected to decrease the cost of drug development, since cardiac toxicity is a major cause of attrition in drug development pipelines. The ability to use a clinically relevant, amenable system to deepen our understanding of cardiac cell biology and drug responses can result in major benefits for the ageing population and economy of developed countries.

Applications

- Assess cardiotoxicity and phenotypic characteristics generated by drugs of interest.
- Model human cardiac development.
- Use as control in studies involving diseased or gene-edited cardiomyocytes.

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 vial of frozen cells	Each vial contains 5 million cells in 1 ml of STEMdiff Cardiomyocyte Freezing Media (Stem Cell Technologies #05030)

Parental Cells

PBMC-derived, non-disease Human iPS Cell Line (XCells 30HU-002)

Mycoplasma Testing

The cells have been screened to confirm the absence of Mycoplasma species.

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.

Materials Required but Not Supplied

These materials are not supplied with the cells but are necessary for cell culture and cellular assays. BPS Bioscience's recommended reagents are validated and optimized for use with these cells 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
Thiazovivin	BPS Bioscience #78506
DMEM/F12	ThermoFisher #11330032
Matrigel™	Corning #354230

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. Cells should be maintained 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 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 Protocol

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

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

A. Validation Data

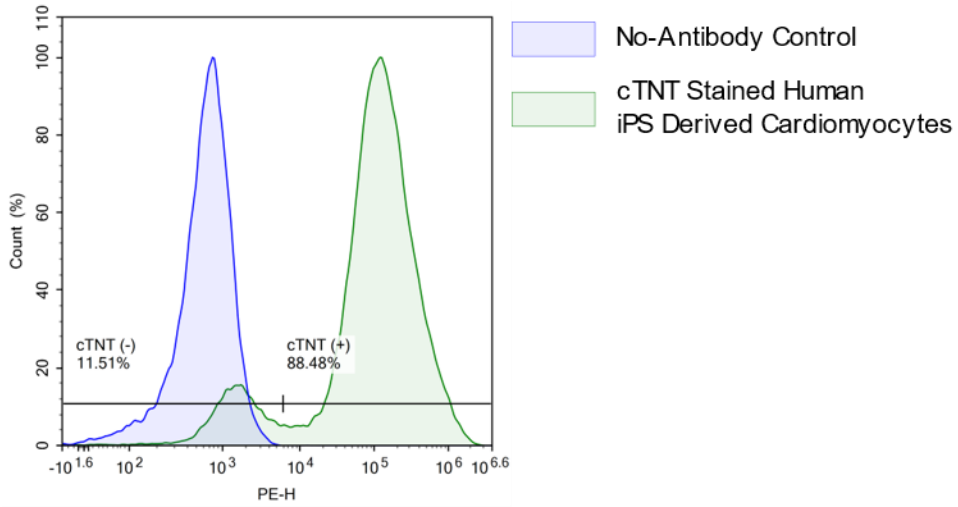

Figure 1. Cardiac Troponin-T (cTNT) expression in Human iPSC Derived Cardiomyocytes by flow cytometry.
Cardiomyocytes were dissociated using TrypanLE™ Select (ThermoFisher #12563011), fixed and intracellularly stained with PE Mouse Anti-Cardiac Troponin T antibody (BD Biosciences # 564767) (green) or no antibody (blue), and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates PE intensity.

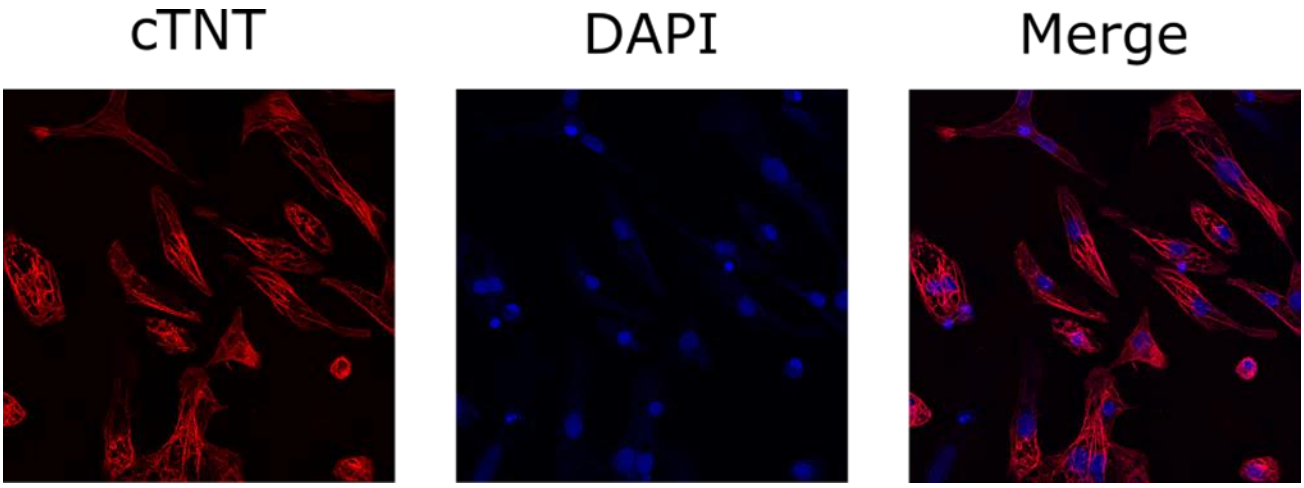

Figure 2. Cardiac Troponin-T (cTNT) expression and localization in Human iPSC Derived Cardiomyocytes by immunofluorescence.
Cardiomyocytes were thawed and plated on Matrigel-coated slides at <75,000 cells/cm^2. Cells were fixed and intracellularly stained for the typical cardiomyocyte marker cTNT using Cardiac Troponin T Monoclonal Antibody (ThermoFisher MA5-12960) followed by Goat Anti-Mouse IgG (H+L), Cross-Adsorbed, DyLight™ 594 antibody (ThermoFisher 35511). Nuclei were counterstained with DAPI. Images were captured using a Zeiss880 confocal microscope.

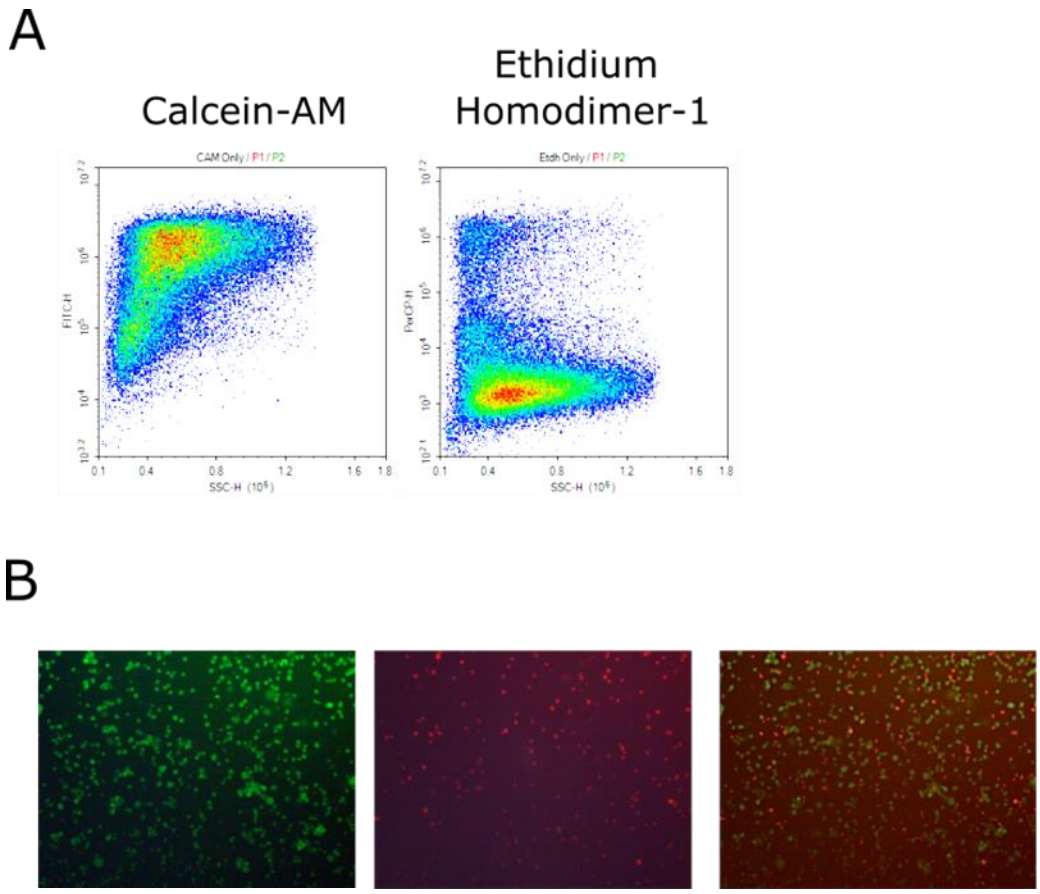

Figure 3. Cell viability and plating efficiency of Human iPSC Derived Cardiomyocytes at thaw. Human iPSC Derived Cardiomyocytes were thawed as described in the above protocol. Cell viability of Human iPSC Derived Cardiomyocytes was measured using Live/Dead Viability/Cytotoxicity Kit for mammalian cells (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. The majority of cells are viable and able to attach after thaw.

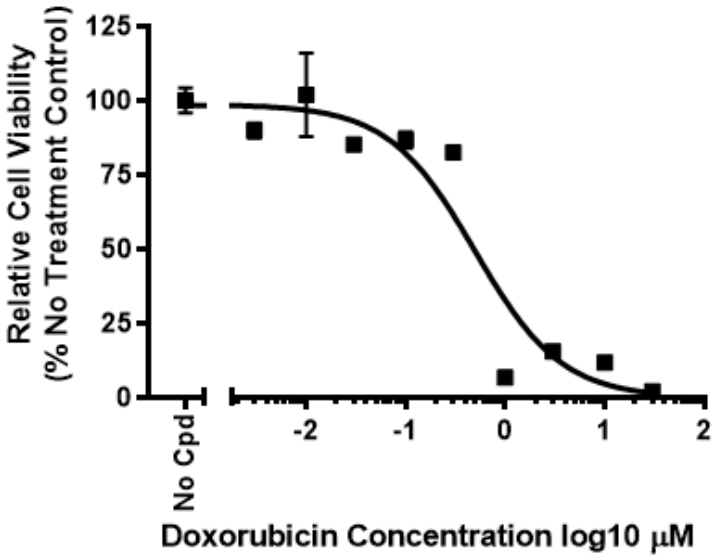

Figure 4. Human iPSC Derived Cardiomyocytes response to the known cardiotoxic drug doxorubicin.
Human iPSC Derived Cardiomyocytes were plated at a density of ~25,000 cells/well on a Matrigel™-coated 96-well plate and allowed to recover for 96 hours. Cardiomyocytes were treated with increasing concentrations of doxorubicin for 72 hours. Cell viability was measured using CellTiter-Glo® Luminescence Cell Viability Assay (Promega #65570).

References

Takahashi K., *et al.,* 2007, *Cell* 131: 861-872.
Yamanaka S., *et al.,* 2012, *Cell Stem Cell* 10: 678-684.
Lian X., *et al.,* 2012, *PNAS* 109(27): E1848-E1857.
Musunuru K., *et al.,* 2018 *Circulation: Genomic and Precision Medicine* 11: e000043.
Mordwinkin., *et al.,* 2013, *Journal of Cardiovascular Translational Research* 6: 22-30.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product. 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. Commercial use of these cells is not allowed. Commercial use requires the appropriate license from iPS Academia Japan, Inc.

This cell line is for research use only, not for therapeutic or prophylactic use in humans or animals. Use in humans is strictly prohibited.

Publications using these cells should reference BPS Bioscience, Inc., San Diego.

Troubleshooting Guide

For further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
TCF/LEF StemBright™ Luciferase iPS Cell Pool (Wnt Pathway)	78515	1 vial
Cas9 Expressing iPS Cell Pool	78578	1 vial
Cas9 Inducible (Tet-On) iPS Cell Pool	78845	1 vial
StemBright™ Luciferase iPS Cell Pool	78594	1 vial

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