Description

The TCF/LEF (T-cell factor/lymphoid enhancing factor) StemBright™ Luciferase Cells contain a firefly luciferase gene under the control of TCF/LEF responsive elements, stably integrated into induced Pluripotent Stem (iPS) cells. TCF/LEF transcription factors are downstream of the Wnt signaling pathway. This cell pool is validated for its response to GSK3β inhibitor CHIR-99021, which activates the Wnt signaling pathway in human pluripotent stem cells.

Background

The Wnt signaling pathway is a well described regulator of organism development and cell fate specification. Cellular pluripotency and differentiation of multiple lineages, including cardiomyocytes and neurons are modulated by this pathway. There are 19 Wnt ligands and 10 Frizzled receptors, which stimulate canonical as well as non-canonical Wnt pathways. In the canonical pathway, cell activation by a Wnt ligand leads to the stabilization and nuclear translocation of β-catenin and subsequent activation of TCF/LEF transcription factor. The Wnt signaling pathway has recently been shown to play a role in cell cycle reentry and proliferation of iPS-derived cardiomyocytes. It is a therapeutic target in the treatment of neuropsychiatric disorders.

Application(s)

Screen for Wnt-activating small molecules in iPS cells

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 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
2 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

Materials Used in the Cellular Assay

Name	Ordering Information
CHIR-99021	BPS Bioscience #27614
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

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.

Stability

As this is a cell pool and not a cell line, BPS Bioscience cannot guarantee the stability of the genetic modifications over time. Clonal derivation may occur. We recommend freezing cell vials very early on and growing the cells for a limited number of passages. Cells should be cultured using Growth Media, which contain selection antibiotics.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be cultured at 37°C 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 the cells.

iPSC Growth Medium:
mTeSR™ Plus supplemented with 1% Penicillin/Streptomycin and 0.5 µg/ml Puromycin.

iPSC Passage Medium:
mTeSR™ Plus supplemented with 1% Penicillin/Streptomycin and 0.5 µg/ml Puromycin and 1 µM Thiazovivin.

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

Media Used in the Functional Cellular Assay

iPSC Thaw Medium:
mTeSR™ Plus supplemented with 1% Penicillin/Streptomycin **(does NOT contain Thiazovivin).**

Cell Culture Protocols

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 all manufacturer's instructions for Matrigel handling. Matrigel should not be subjected to repeated freeze/thaw cycle.

When first using a vial of Matrigel, it is recommended to aliquot into microcentrifuge tubes at volumes of ~100 µl and/ or ~200 µl per vial for future use.

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

2. Gently 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 shows example volumes used for 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
4 x T25 Flask	~100 µl	12 ml	3 ml/Flask
3 x T75	~200 µl	30 ml	10 ml/Flask
2X T175	~ 300 µl	40 ml	20 ml/Flask

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.**

   Matrigel-coated plates can be stored in the CO_2 Incubator at 37°C for 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. Equilibrate the Thaw Medium (no Puromycin) to room-temperature. **iPSC Thaw Medium should NOT be pre-warmed in a water bath.**

3. Prepare 15 ml of Complete iPSC Thaw Medium by adding Thiazovivin at 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 in dry ice if the transfer to culture will take longer than a minute. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the 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. **Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.**

5. Using a 10 ml serological pipette, slowly add 10 ml of Complete iPSC Thaw Medium (contains Thiazovivin, no Puromycin) to the conical tube containing the iPS cells. The Thaw Medium should be added dropwise while softly rocking the conical tube to permit gentle mixing and avoid osmotic shock.

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

7. Aspirate the DMEM/F12 medium covering the Matrigel from 2 wells of a 6-well Matrigel-coated plate.

8. 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.

9. After 24 hours of culture, check for cell attachment and viability. Change the culture medium to fresh Thaw Medium (no Puromycin or Thiazovivin) and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

10. Cells should be passaged before they are 85% confluent or before colonies become too large, whichever comes first. At first passage and subsequent passages, use iPS Cell Growth Medium **(contains Puromycin)**.

11. Perform media changes as recommended in the typical cell maintenance schedule below. For the TCF/LEF StemBright™ Luciferase iPS Cell Pool cultivated in mTeSR Plus, we recommend one media change on either Saturday or Sunday. Individual results may vary.

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 the Matrigel-coated culture plate or flask at least 1 hour in advance.

3. Prepare 15 ml of Passage Medium by adding 1.5 µl of 10 mM Thiazovivin stock to 15 ml of Growth Medium (contains Puromycin).

4. Aspirate the cell culture medium, and gently wash the cells with phosphate buffered saline (PBS).

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 (contains Thiazovivin and Puromycin), 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 DMEM/F12 media overlay from Matrigel-coated plates before plating the cells.

Cell Freezing

The Medium used in this protocol contains Thiazovivin but does not contain Puromycin and is the same as *Complete iPSC Thaw Medium.*

1. Add 1.5 µl of 10 mM Thiazovivin stock to 15 ml of iPSC Thaw Medium to make Complete iPSC Thaw Medium.

2. Prepare 2X Freezing Medium: 80% Medium with 1 µM thiazovivin prepared above + 20% DMSO.

3. Aspirate the cell culture medium and wash the cells with phosphate buffered saline (PBS).

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™, leaving a very thin film.

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 medium and resuspend the cells in Complete iPSC Thaw Medium using 0.5 ml of medium per freezing vial.

8. Using a 10 ml serological pipette, slowly add an equal volume of 2X Freezing Medium (0.5 ml per freezing vial, contains Thiazovivin and 20% DMSO) to the conical tube containing the iPS Cells. The 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 aliquots into cryogenic vials. 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 storage.

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

Validation Data

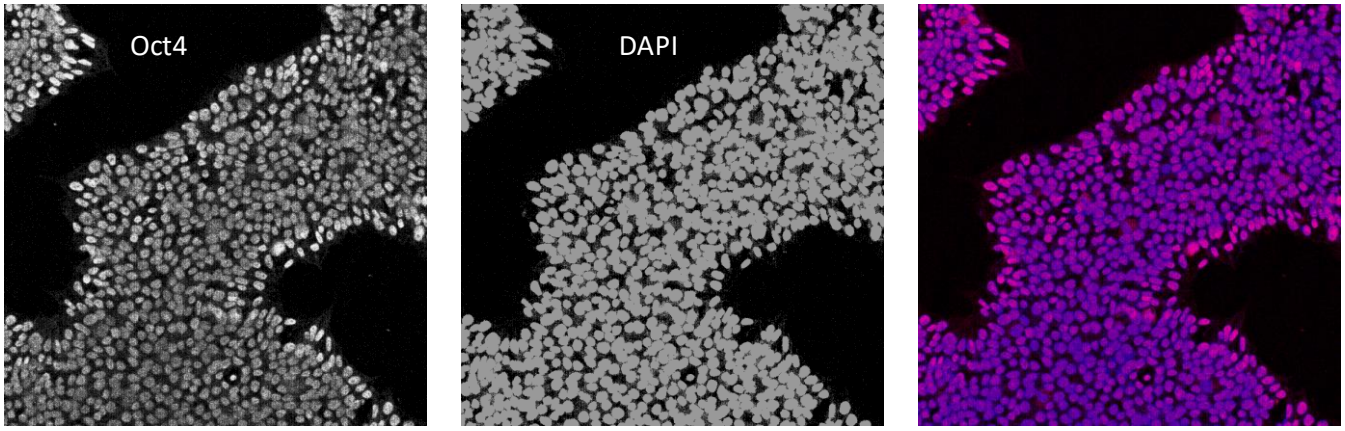

Figure 1. Immunofluorescence staining of TCF/LEF StemBright™ Luciferase iPS Cell Pool (Wnt Pathway) for Oct-4.
TCF/LEF StemBright™ Luciferase iPS Cell Pool (Wnt Pathway) were plated on Matrigel-coated slides using routine passaging with RelesR. Cells were fixed and stained for iPS Cell marker Oct-4 using anti-Oct-4 antibody (Biolegend #653701) and Dylight-594 conjugated anti-mouse secondary (Thermo Fisher #35511). Nuclei were counterstained with DAPI. Slides were imaged on a Zeiss880 confocal microscope. The merge of Oct-4 and DAPI is shown in purple (right panel).

A. Functional characterization of the TCF/LEF StemBright™ Luciferase iPS Cell Pool (Wnt Pathway).
The following assays were designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.

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

a. Dose response of GSK3-β inhibitor CHIR-99021 in TCF/LEF StemBright™ Luciferase iPS Cell Pool.

1. Ensure that you have prepared a Matrigel™-coated, clear-bottom white 96-well plate at least 1 hour in advance (can be prepared up to two weeks in advance).

2. Prepare 15 ml of iPSC Passage Medium by adding 1.5 µl of 10 mM Thiazovivin stock to 15 ml Growth Medium (contains Puromycin).

3. Aspirate the cell culture medium, wash the cells with phosphate buffered saline (PBS).

4. Add 1 ml of Accutase in each well of a 6-well plate and rock to evenly distribute.

5. Incubate at room temperature for 5-10 minutes or until colonies have fully lifted from the plate and have begun to dissociate into single cells.

6. Once the cells have detached, add iPSC Passage Medium (contains Thiazovivin and Puromycin), transfer to a 50 mL conical tube and centrifuge at 300 x g for 5 minutes.

7. Aspirate the supernatant, count the cells, and resuspend in iPSC Passage Medium.

8. Seed cells at 40,000 cells/100 µl/well in a Matrigel™-coated, clear-bottom white 96-well plate. (Be sure to aspirate the DMEM/F12 media overlay from Matrigel coated plates before plating the cells).

9. Allow cells to attach for 24 hours and change the cell culture medium to fresh iPSC Growth Medium (no Thiazovivin, contains Puromycin).

10. Allow cells to expand for 24 hours.

11. Prepare a serial dilution of GSK3-β inhibitor CHIR-99021, which activates Wnt signaling, in iPSC Thaw Medium (no Thiazovivin or Puromycin).

12. Aspirate the culture medium from the culture plate.
    - Add 100 µl of CHIR-99021 serial dilution to wells labeled as “stimulated”.
    - Add 100 µl of iPSC Thaw Medium to the “unstimulated control” wells.
    - Add 100 µl of iPSC Thaw Medium to cell-free control-wells (for determining background luminescence).

13. Incubate the plate at 37°C in a CO_2 incubator for 24 hours.

14. Perform a luciferase assay using ONE-Step™ Luciferase Assay buffer: Add 100 µl of ONE-Step™ Luciferase Assay buffer per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.

15. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The Fold induction of TCF/LEF luciferase reporter expression is the background-subtracted luminescence of CHIR-99021-treated well divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ Induction = \frac{lumin.treated\ cells - ave.background\ lumin.}{ave.lumin.\ untreated\ cells - ave.background\ lumin.}$$

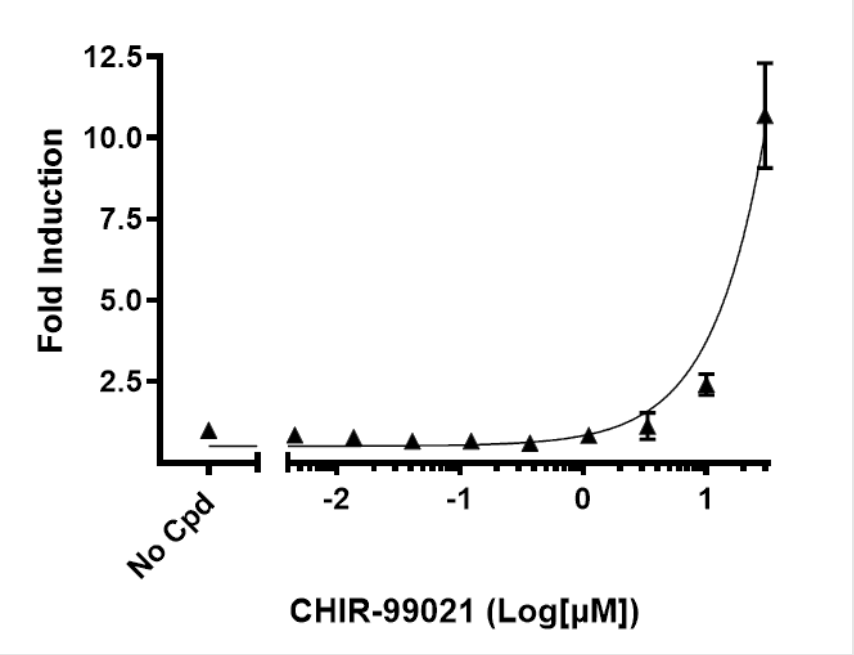

Figure 2: Dose response of GSK3-β inhibitor CHIR-99021 in TCF/LEF StemBright™ Luciferase iPS Cell Pool.
Cells were treated with increasing doses of GSK3-β inhibitor CHIR-99021, which stimulates the TCF/LEF transcription factor, for 24 hours. Luciferase activity was measured using the ONE-Step Luciferase Assay System. Results are expressed as fold induction of TCF/LEF luciferase reporter activity.

References

Buikema J, *et al.* (2020) *Cell Stem Cell* **27(1):** 50-63.
Clevers, H. (2006) *Cell* **127(3):**469-480
Lian X, *et al.* (2013) *Nat Protoc.* **8(1):**162-175

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.

The purchase of this cell line grants you a 10-year license to use this cell line in your immediate laboratory for internal research purposes. Commercial use of this cell line 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.

This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit modification of the cell line in any way. Modification of this cell line or transfer to another facility of the cells requires a separate license or additional fees; contact sales@bpsbioscience.com for details. Publications using this cell line should reference BPS Bioscience, Inc., San Diego.

Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory. BPS does not warrant the suitability of the cell line for any particular use and does not accept any liability in connection with the handling or use of the cell line. Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
TCF/LEF Luciferase Reporter Lentivirus (Wnt/β-catenin Signaling Pathway)	79787	500 µl x 2
TCF/LEF Reporter HEK293 Cell Line (Wnt Signaling Pathway)	60501	2 vials
Wnt-C59	27780	Various Sizes
Non-Diseased Human iPSC-derived Cardiomyocytes	78437	Various Sizes