SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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

HRE Luciferase Reporter ME-180 Cell Line is designed to monitor the activity of hypoxia-inducible factors (HIFs). It contains a firefly luciferase reporter driven by multiple copies of hypoxia response element (HRE) located upstream of the minimal TATA promoter. As oxygen becomes rate limiting, HIFs form heterodimers that recognize cognate HREs, inducing transcription of the luciferase reporter.

The cell line has been functionally validated in response to the hypoxia-mimetics cobalt chloride ($CoCl_2$) and Deferoxamine (DFO).

Background

Hypoxia occurs in solid tumors as a result of poor vascularization within the core of the tumor. It is a driver of tumor progression and resistance to therapy through adaptive responses. Hypoxia response elements (HREs) are transcription factor binding sites within the promoters of various genes regulated by hypoxia-inducible factors (HIFs). As oxygen becomes rate limiting, HIFs form heterodimers that recognize cognate HREs, thus activating the transcription of genes involved in cell proliferation, metastasis, and angiogenesis. HIF activation in many types of cancer correlates with poor outcomes. The study of these pathways may open new therapeutic avenues for cancer treatment.

Application

- Study hypoxia-related signaling pathways

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Host Cell

ME-180 is a human cancer cell line exhibiting epithelial morphology that was isolated from the uterus of a female patient with epidermoid carcinoma, adherent.

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 this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems 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.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1Q	BPS Bioscience #78096

BPS Bioscience ● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

Materials Required for Cellular Assays

Name	Ordering Information
$CoCl_2$	Sigma #C8661
Deferoxamine	Selleck Chem #S5742
Assay Medium: Assay Medium 1C	BPS Bioscience #78674
96-well tissue culture-treated white clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells will arrive 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: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1Q (BPS Bioscience #78096):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin and 1 µg/ml of Puromycin.

Media Required for Functional Cellular Assay

Assay Medium:

Assay Medium 1C (BPS Bioscience #78674):

MEM medium supplemented with 0.5% charcoal stripped FBS, 1% non-essential amino acids, 1 mM Na pyruvate.

Cell Culture Protocol

Note: ME-180 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), Transfer the contents of the cryovial to a tube containing 10 ml of Thaw Medium 1.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell viability and attachment. For a T25 flask, add 3-4 ml of Thaw Medium 1, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they reach full confluency. Switch to Growth Medium 1Q at first and subsequent passages.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1Q and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1Q.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10-1:20 once or twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+} and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1Q and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. 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.

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

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

Validation Data

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- Assay should include "Stimulated", "Background Control" and "Unstimulated Control" conditions.

1. Seed HRE Luciferase ME-180 cells at a density of 20,000 cells per well into a white, clear-bottom 96-well culture plate in 100 µl of Thaw Medium 1. Leave empty wells as cell-free control wells ("Background Control").

2. Incubate cells at 37°C with 5% CO_2 overnight.

3. Prepare a two-fold serial dilution of agonist in Assay Medium 1C (100 µl/well).

4. Remove the medium from the wells.

5. Add 100 µl of each dilution to the wells labeled as "Stimulated".

6. Add 100 µl of Assay Medium 1C to the "Unstimulated Control" wells (for measuring uninduced level of HRE reporter activity).

7. Add 100 µl of Assay Medium 1C to "Background Control" wells.

8. Incubate at 37°C with 5% CO_2 overnight (18-22 hours).

9. The expression of luciferase can be measured using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690).

10. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of HRE luciferase reporter expression is the background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

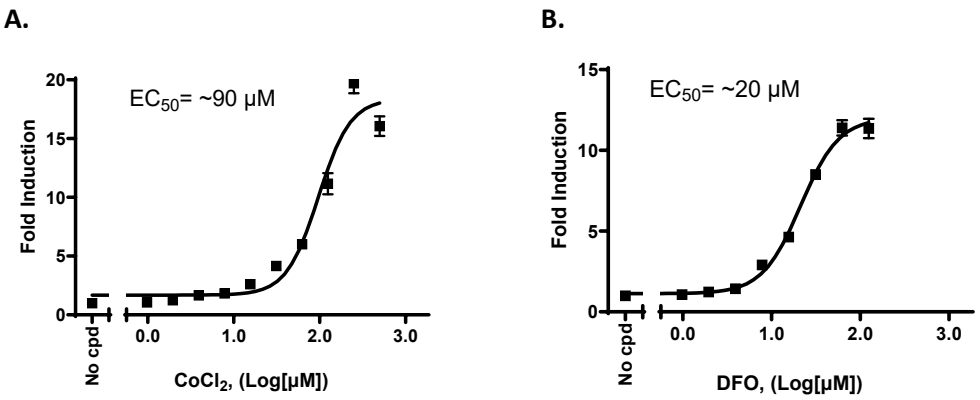

Figure 1: HRE Luciferase Reporter activity stimulated by $CoCl_2$ and Deferoxamine.
HRE Luciferase reporter ME-180 cells were treated with increasing doses of $CoCl_2$ (A) or Deferoxamine (B) for 20 hours and luciferase activity was measured using the ONE-Step™ Luciferase Assay System. Results are shown as fold induction of luciferase reporter expression.

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

References

Xia, et al; Molecular Cancer 2009, **8**:117

License Disclosure

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*
HRE Luciferase Reporter Lentivirus	78668	500 µl x2

Version 030325