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NFAT Luciferase Reporter CHO Cell Line (PKC/Ca^{2+} Signaling Pathway)

#78569

Description

The NFAT Luciferase Reporter CHO cell line (PKC/Ca2+ Signaling Pathway) contains a firefly luciferase gene under the control of NFAT response element stably integrated into CHO K1 cells. This cell line is validated for its response to phorbol 12-myristate 13-acetate (PMA) in the presence of ionomycin.

Background

The protein kinase C (PKC)/ Ca^{2+} response pathway leads to activation of the transcription factor nuclear factor of activator T cells (NFAT). NFAT is regulated by Ca^{2+} and the Ca^{2+}/calmodulin-dependent serine phosphatase calcineurin. NFAT proteins are phosphorylated and reside in the cytoplasm in resting cells; upon stimulation, they are dephosphorylated by calcineurin, translocate to the nucleus, and induce gene expression.

Application(s)

- Monitor NFAT stimulation
- Screen for activators or inhibitors of the PKC/ Ca^{2+} pathway.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience, #79796)

Parental Cell Line

CHO-K1 cells, Chinese Hamster Ovary, epithelial-like cells, 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 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 3	BPS Bioscience #60186
Growth Medium 3D	BPS Bioscience #79539

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, 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 grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 3 (BPS Bioscience #60186):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3D (BPS Bioscience #79539):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin.

Materials Required for Cellular Assay

Name	Ordering Information
Assay Medium: Thaw medium 3	BPS Bioscience #60186
Growth Medium 3D	BPS Bioscience #79539
PMA	LC Laboratories #P1680
Ionomycin	Sigma #I3909
96-well tissue culture treated white clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Cell Culture Protocol

Cell Thawing

1. 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 a tube containing 10 ml of pre-warmed Thaw Medium 3 (**no Geneticin)**.
   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 3 (**no Geneticin)**.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 (**no Geneticin)** 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 are fully confluent. At first passage and subsequent passages, use Growth Medium **3D containing Geneticin (G418)**.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 3D and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium **3D (contains Geneticin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:10 to 1:20 weekly or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium **3D** 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.

4. 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.

5. 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

- The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volumes should be scaled appropriately.
- The experiment should be performed in triplicates.

Response of NFAT Luciferase Reporter CHO cells to PMA and Ionomycin

1. Seed NFAT Luciferase Reporter CHO cells at a density of ~32,000 cells per well into white clear-bottom 96-well cell culture plate in 90 µl of Thaw Medium 3.

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

3. Dilute PMA with ionomycin into Thaw Medium 3 at concentrations that are 10-fold higher than the desired final concentrations. Add 10 µl of the dilutions to the "stimulated" wells.

4. Add 10 µl of Thaw Medium 3 to the "unstimulated control" wells (for measuring uninduced level of NFAT reporter activity)

5. Add 100 µl of Thaw Medium 3 to cell-free control wells (for determining background luminescence).

6. Incubate the cells at 37°C in a 5% C O_2 incubator overnight (~18 hours).

7. The next day, Prepare ONE-Step™ Luciferase Assay reagent according to recommended instructions. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for 15 to 30 minutes and measure luminescence using a luminometer.

Results

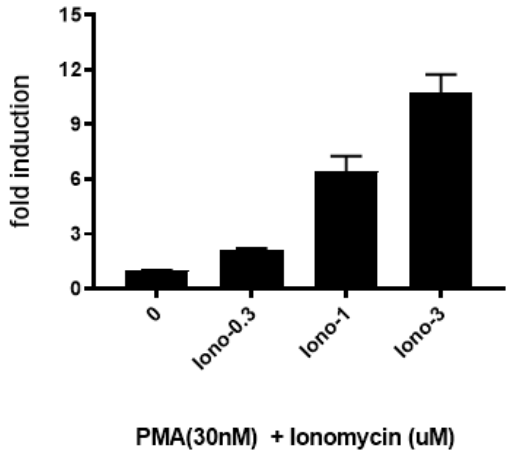

Figure 1. NFAT Luciferase Reporter CHO cells response to Ionomycin with PMA.
The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for unstimulated control cells.

References

Rinne, A. *et al*, (2009). Journal of Molecular and Cellular Cardiology, 47**(3)**, 400–410.

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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*
NFAT Luciferase-eGFP Reporter Jurkat cell line	78662	2 vials
TIGIT / NFAT Reporter - Jurkat Cell Line	60538	2 vials
PD-1 / NFAT Reporter - Jurkat Recombinant Cell Line	60535	2 vials
LAG3 / NFAT Reporter - Jurkat Recombinant Cell Line	71278	2 vials
NFAT Reporter (Luciferase) – THP-1 Cell Line	78320	2 vials
NFAT Reporter (Luc) – Jurkat Recombinant Cell Line	60621	2 vials