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NFAT Luciferase Reporter RBL-2H3 Cell Line (PKC/Ca^{2+} Signaling Pathway)

#83571

Description

The NFAT Luciferase Reporter RBL-2H3 Cell Line (PKC/Ca^{2+} Signaling Pathway) is a rat-derived basophilic leukemia RBL-2H3 cell line that contains a firefly luciferase reporter under the control of NFAT (nuclear factor of activator T cells) response element stably integrated into RBL-2H3 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.

RBL-2H3 is a rat basophilic leukemia cell line that may be a valuable model for IgE-mediated degranulation of the mast cells.

Application(s)

- Monitor NFAT stimulation in RBL-2H3 cells.
- Screen for activators or inhibitors of the PKC/ Ca^{2+} pathway.

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)

Parental Cell Line

RBL-2H3, Rat, Basophilic Leukemia, 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 6	BPS Bioscience #60183
Growth Medium 6J	BPS Bioscience #83594

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 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 6 (BPS Bioscience #60183):
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 6J (BPS Bioscience #83594):
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 500 µg/ml of Geneticin.

Materials Required for Cellular Assay

Name	Ordering Information
Assay Medium: Thaw Medium 6	BPS Bioscience #60183
Growth Medium 6J	BPS Bioscience #83594
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 6.

   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 6.

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 6 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 6J.

Note: When RBL-2H3 cells are confluent some cells are likely to detach and float. That is normal and you can remove the floating cells and wash with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+} as in the Cell Passage step 1 described below).

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 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 6J 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 6J.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10 every three days.

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 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 6J 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 ~1 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 at least 10 vials at an early passage for future use.

Validation Data

- The following assay is 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 using triplicates.
- The assay should include “Stimulated”, “Unstimulated Control” and “Cell-Free Control” conditions.

Response of NFAT Luciferase Reporter RBL-2H3 Cell Line to PMA and Ionomycin

1. Seed NFAT Luciferase Reporter RBL-2H3 cells at a density of 30,000 ~ 40,000 cells per well into white clear-bottom 96-well cell culture plate in 90 µl of Thaw Medium 6. Leave a few empty wells as “Cell-Free Control”.

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

3. Dilute PMA with ionomycin into Thaw Medium 6 at concentrations that are 10-fold higher than the desired final concentrations (10 µl/ well).

4. Add 10 µl of the dilutions to the "Stimulated" wells.

   Note: Excess amount of DMSO is toxic to the cells. Keep the final DMSO concentration lower than 0.2% if PMA and/or Ionomycin stock solutions were prepared in DMSO.

5. Add 10 µl of Thaw Medium 6 to the "Unstimulated Control" wells (for measuring uninduced level of NFAT reporter activity)

6. Add 100 µl of Thaw Medium 6 to "Cell-Free Control" wells (for determining background luminescence).

7. Incubate the cells at 37°C in a 5% CO_2 incubator for ~ 5 hours.

8. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well.

9. Incubate at room temperature for 15 - 30 minutes and measure luminescence using a luminometer.

Results

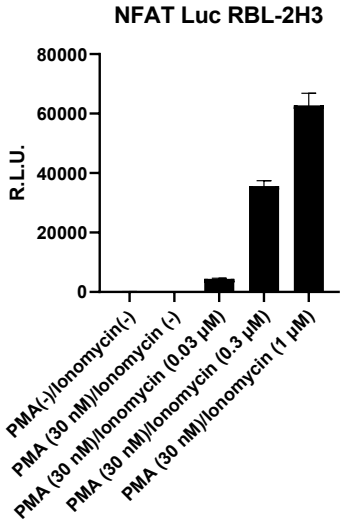

Figure 1. NFAT Luciferase Reporter RBL-2H3 cells response to Ionomycin with PMA.
NFAT Luciferase Reporter RBL-2H3 cells were incubated with a 30 nM PMA and different amounts of Ionomycin. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690). Unstimulated cells were used as control.

Data shown is representative.

References

Passante E.*, et al*, 2009 *Inflamm. Res.* 58(11): 737-745.

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

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

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