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Produktinformation

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

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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

The NFAT reporter (Luciferase)-THP-1 cell line is designed for monitoring the NFAT (nuclear factor of activated T-cells) signaling pathway in THP-1 cells by measuring luciferase activity. It contains a firefly luciferase gene driven by the NFAT response element located upstream of the minimal TATA promoter. Upon activation by NFAT activators such as Ionomycin, endogenous NFAT transcription factors bind to the DNA response elements, inducing transcription of the luciferase reporter gene.

Application

- Monitor NFAT signaling pathway activity.
- Screen for compound activity on the NFAT signaling 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)

Host Cell

THP-1 Human leukemia monocytic cell line. Non-adherent cells.

Mycoplasma Testing

The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) 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 2	BPS Bioscience, #60184
Growth Medium 2M	BPS Bioscience, #78181

Materials Required for Cellular Assay

Name	Ordering Information
Ionomycin	Sigma, #I3909
PMA	Sigma-Aldrich, #P8139
Cyclosporin A	Cayman, #12088
Assay Medium: Thaw Medium 2	BPS Bioscience, #60184
Growth Medium 2M	BPS Bioscience, #78181
96-well tissue culture treated white clear-bottom assay plate	Corning, #3610
ONE-Step™ luciferase assay system	BPS Bioscience, #60690
Luminometer	

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

Storage Conditions

Cells will arrive upon 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. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2 using Growth Medium 2M.

Media Required for Cell Culture

Thaw Medium 2 (BPS Bioscience, #60184):
RPMI1640 medium (Life Technologies, #A10491-01) supplemented with 10% FBS (Life Technologies, #26140-079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

Growth Medium 2M (BPS Bioscience, #78181):
Thaw Medium 2 (BPS Bioscience, #60184) plus 1 µg/ml of Puromycin (Invivogen, #ant-pr-1).

Assay Medium: Thaw Medium 2 (BPS Bioscience, #60184)

Cell Culture Protocol

1. It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 (**no Puromycin**).
2. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 2 (**no Puromycin**).
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, add an additional 1 – 2 ml of Thaw Medium 2 (**no Puromycin**).
5. At first passage, switch to Growth Medium 2M (**contains Puromycin**).
6. To passage the cells, dilute cell suspension into new culture vessels at no less than 0.5 x 10^6 cells/ml. Do not allow the cell density to exceed 2.0 x 10^6 cells/ml.

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

Assay Performance

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

A. NFAT signaling activation by Ionomycin and Ionomycin/PMA

1. Harvest NFAT reporter (Luciferase)-THP-1 cells from culture in Growth Medium 2M and seed cells at a density of 45,000 cells per well into white opaque 96-well microplate in 50 µl of assay medium.
2. Prepare an intermediate solution of the compound (ionomycin or PMA with ionomycin) by diluting it into assay medium at 2x desired final concentration. Add 50 µl of diluted compound to each well for a final concentration of 1x in 100 µl. Note: The final DMSO concentration can be up to 0.5%.
3. Add 50 µl of assay medium with same concentration of DMSO but without the activator (Ionomycin or Ionomycin/PMA) to the unstimulated control wells.
4. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
5. Incubate at 37°C with 5% CO_2 for 5-6 hours.
6. Prepare ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100 µl of ONE-Step™ Luciferase reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all readings.

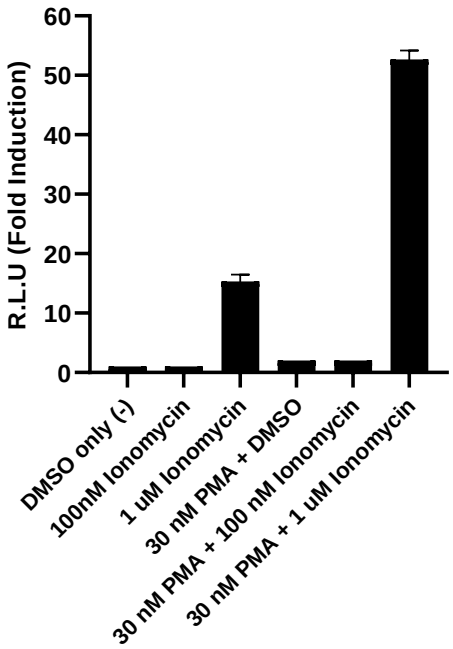

Figure 1. **NFAT activation in NFAT reporter (Luciferase)-THP-1 cells.**
The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without Ionomycin/PMA.

B. Testing inhibitors

1. Harvest NFAT reporter (Luciferase)-THP-1 cells from culture in Growth Medium 2M and seed cells at a density of 40,000 cells per well into white opaque 96-well microplate in 50 µl of assay medium.
2. Prepare serial dilutions of test compounds or Cyclosporin A at 10x desired concentration in assay medium. Add 10 µl of serially diluted test compounds to the cells.
3. Add 10 µl of assay medium containing the same concentration of DMSO to the positive control wells (Positive control is stimulated by PMA/Ionomycin but does not contain inhibitor, for measuring uninhibited level of NFAT reporter activity after stimulation – 100% activity) and to the negative control wells (Negative control is not stimulated by PMA/Ionomycin, for measuring unstimulated level of NFAT reporter activity – 0% activity).
4. Incubate at 37°C with 5% CO_2 for 1 hour.
5. Add 50 µl of assay medium containing 2 µM Ionomycin and 60 nM PMA to all wells except the negative control wells (final concentrations of Ionomycin and PMA are 1 µM and 30 nM respectively). Add 50 µl of assay medium containing the same amount of DMSO to the negative control wells.
6. Incubate at 37°C with 5% CO_2 for 5 – 6 hours.
7. Prepare ONE-Step™ Luciferase Assay reagent per 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. Subtract background luminescence value from all readings.

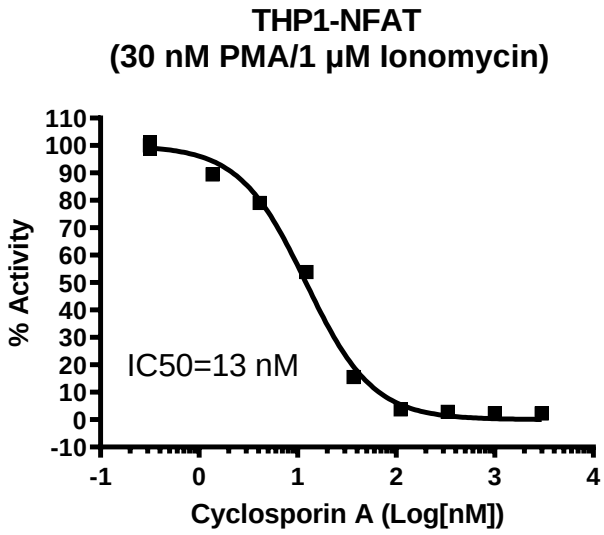

Figure 2. **Cyclosporin A inhibits NFAT signaling pathway activated by Ionomycin/PMA.**
Percent activity was determined by comparing luminescence values against the mean value for the positive control cells.

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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.

References

1. Clipstone NA, Crabtree GR. *Nature*. 1992 Jun 25;**357(6380):**695-7.
2. Lyakh, L., *et al. Mol Cell Biol.* 1997 May;**17(5):**2475-84.

Related Products

Products	*Catalog #*	*Size*
NFAT Reporter (Luc) – Jurkat Recombinant Cell Line	60621	2 vials
NFAT Reporter – Hek293 Cell Line (PKC/ Ca2+ Pathway)	79298	2 vials
NF- κB Reporter (Luc) – THP-1 Cell Line	79645	2 vials
NF-κB Reporter (Luc) - CHO-K1 Cell Line	60622	2 vials
NF-κB Reporter (Luc) - Jurkat Cell Line	60651	2 vials
ONE-Step™ Luciferase Assay System	60690	Multiple sizes
Thaw Medium 2	60184	100 ml
Growth Medium 2M	78181	500 ml
NFAT Luciferase Reporter Lentivirus	79579	500 µl x 2
NFAT eGFP Reporter Lentivirus	79922	500 µl x 2