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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- 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

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Description

The Muscarinic Acetylcholine Receptor (mAChR) M3/NFAT Luciferase Reporter HEK293 Cell Line is a HEK293 cell line expressing the full-length human muscarinic acetylcholine receptor M3 (CHRM3/M3R/M3; accession number: NM_000740.3) and a firefly luciferase reporter under the control of the NFAT (nuclear factor of activated T cells) response element.

This cell line has been validated by flow cytometry and for its responses to the muscarinic acetylcholine receptor agonists Oxotremorine M and Carbamoylcholine chloride (carbachol). Luciferase activity increases in proportion to mAChR activation.

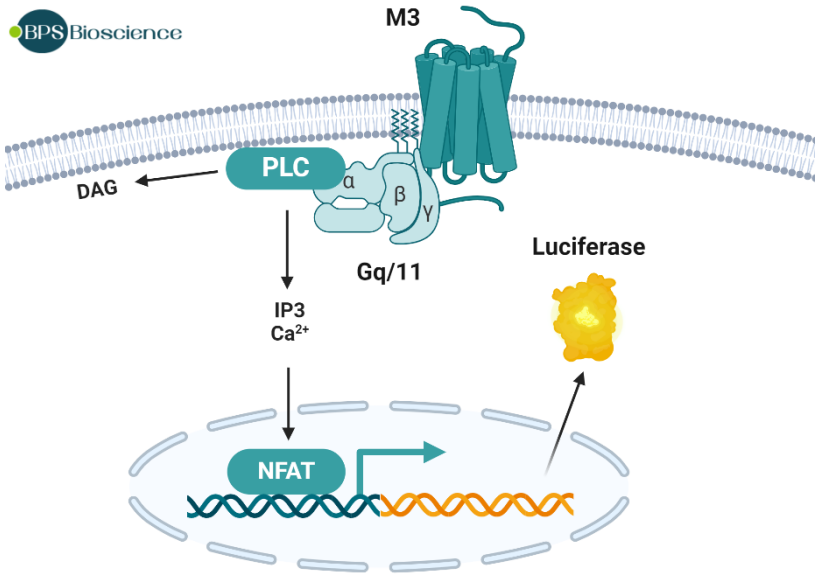

Figure 1: Diagram illustrating the mechanism of action of Muscarinic Acetylcholine Receptor (mAChR) M3/NFAT Luciferase Reporter HEK293 Cell Line.

Background

Muscarinic acetylcholine receptors (mAChRs) are relatively abundant and mediate many of the diverse actions of acetylcholine in the CNS (central nervous system), as well as throughout non-nervous tissues innervated by the parasympathetic nervous system. They are involved in regulating a large number of cognitive, behavioral, motor, and autonomic functions. There are five muscarinic receptor subtypes referred to as M1, M2, M3, M4 and M5 which belong to the GPCR (G-protein-coupled receptor) superfamily. M1, M3 and M5 receptor subtypes couple efficiently through Gq/11, to activate phospholipase C (PLC), which initiates the phosphatidylinositol (IP3) turnover response and leads to the release of intracellular calcium ions (Ca^{2+}). The M2 and M4 receptor subtypes preferentially couple to Gi/o G-proteins and inhibit adenylyl cyclase (AC) activity, which leads to a decrease in the level of cAMP. M2 and M4 receptors also activate G protein-coupled potassium channel by βγ-dimer dissociated from the active Gi/o G-protein, which leads to hyperpolarization of the plasma membrane of excitable cells.
The disruption of muscarinic signaling frequently contributes to several pathophysiological conditions in the CNS and in the periphery. Thus, muscarinic agonists have a wide therapeutic potential in the treatment of neurodegenerative and neuropsychiatric disorders and conditions, e.g. Alzheimer's disease, schizophrenia, pain, and ischemia or heart failure. To target these diseases, selective modulation of individual muscarinic receptor subtypes is necessary to avoid undesired side effects. The orthosteric binding site of all subtypes of muscarinic receptors is virtually the same and no affinity-based selective agonists of muscarinic receptors have been

discovered so far. The development of biased agonists can be the right approach to achieve selective modulation of individual subtypes of muscarinic receptors.

Application

- Screen for activators or inhibitors for M3 receptor-related research and drug discovery.
- Counter-screen functionally selective agonists or biased agonists for M1 and M5 receptors.
- Characterize mAChR M3 antibodies/antagonists and agonists of binding assay.

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

HEK293, 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 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 1A	BPS Bioscience #79528

Materials Required for Cellular Assays

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1A	BPS Bioscience #79528
MEM medium	Hyclone #SH30024.01
Carbamoylcholine chloride	TOCRIS #2810
Oxotremorine M	TOCRIS #1067
Human CHRM5 Antibody	R&D SYSTEMS #MAB 10323
Human CHRM3 antibody	R&D SYSTEMS #MAB 6378
PE Goat anti-mouse IgG (minimal x-reactivity) Antibody	BioLegend #405307
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 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, 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 do *not* contain selective antibiotics. However, Growth Media *do* 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℃ 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 (#60187):

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

Growth Medium 1A (#79528):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 100 µg/ml Hygromycin B and 400 µg/ml Geneticin®, G418 Sulfate.

Validation

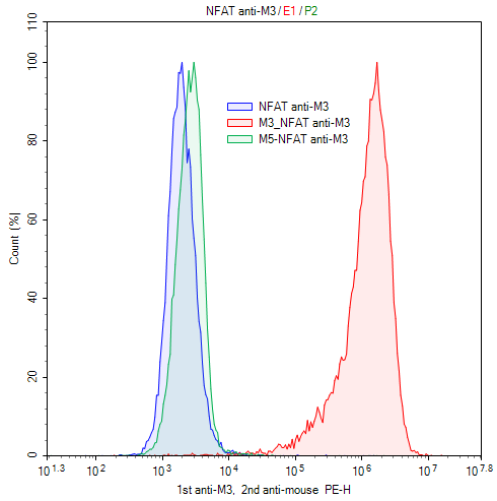

Figure 2. Flow cytometry analysis of the cell surface expression of human mAChR M3 in Muscarinic Acetylcholine Receptor (mAChR) M3/NFAT Luciferase Reporter HEK293 Cell Line.

Muscarinic Acetylcholine Receptor (mAChR) M3/NFAT Luciferase Reporter HEK293 cells, Muscarinic Acetylcholine Receptor (mAChR) M5/NFAT Luciferase Reporter HEK293 cells (#82729) and NFAT Reporter HEK293 control cells (#79298) were stained with Human CHRM3 Antibody, followed by PE-labeled Goat anti-mouse IgG (minimal x-reactivity) Antibody, and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of PE.

Functional Validation

Muscarinic Acetylcholine Receptor (mAChR) M3/NFAT Luciferase Reporter HEK293 Cell Line Agonist Response Assay

- 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", "Unstimulated" and "Background Luminescence" conditions.

1. Harvest Muscarinic Acetylcholine Receptor (mAChR) M3/NFAT Luciferase Reporter HEK293 cells from culture in Growth Medium 1A and seed cells at a density of ~32,000 cells per well into a white clear-bottom 96-well plate in 90 µl of Thaw Medium 1. Leave a few empty wells as "Background Luminescence" control.

2. Incubate the plate at 37°C in a CO_2 incubator for 16 to 24 hours.

3. Prepare a three-fold serial dilution at 10x the final concentrations of Oxotremorine M and Carbamoylcholine chloride agonists separately in Thaw Medium 1 (10 µl/ well). For an EC_{50} dose curve, we recommend a range of approximately 0.002 to 100 µM, final concentration.

4. Add 10 µl diluted Oxotremorine M or Carbamoylcholine to the "Stimulated" wells.

5. Add 10 µl Thaw Medium 1 to "Unstimulated" wells.

6. Add 100 µl Thaw Medium 1 to "Background Luminescence" wells (for determining background luminescence).

7. Incubate the plate at 37°C in a CO_2 incubator for about 5 hours.

8. Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at Room Temperature (RT) for 15-30 minutes.

9. Measure luminescence using a luminometer.

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

$$Fold\ induction = \frac{(luminescence\ of\ stimulated\ cells - avg\ background)}{(avg\ luminescence\ of\ unstimulated\ cells - avg\ background)}$$

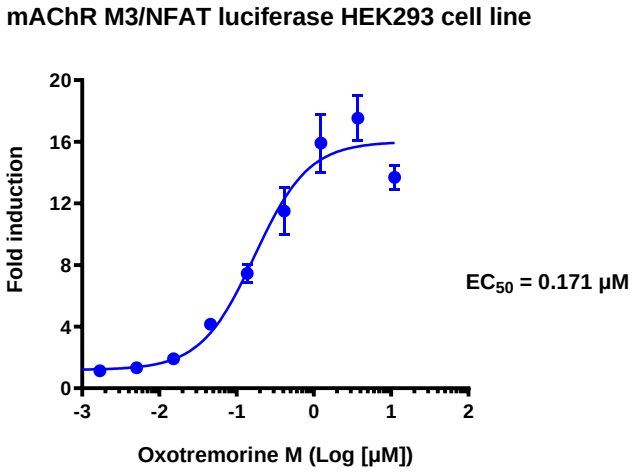

Figure 3. Dose response of Muscarinic Acetylcholine Receptor (mAChR) M3/NFAT Luciferase Reporter HEK293 Cell Line to Oxotremorine.
Oxotremorine M agonist was diluted and added to Muscarinic Acetylcholine Receptor (mAChR) M3/NFAT Luciferase Reporter HEK293 cells for about 5 hours at 37°C in a cell culture incubator. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690).

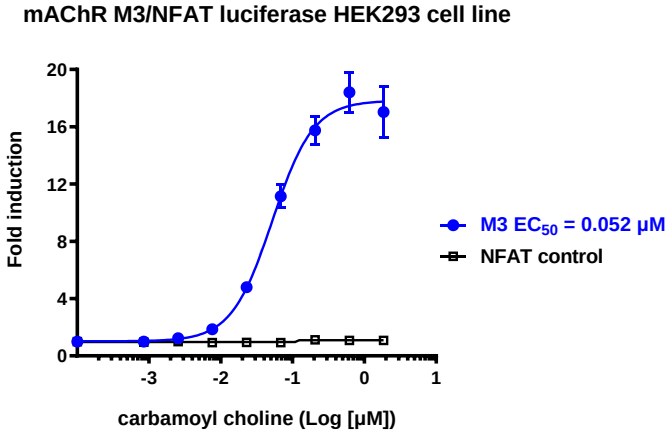

Figure 4. Dose response of Muscarinic Acetylcholine Receptor (mAChR) M3/NFAT Luciferase Reporter HEK293 Cell Line to carbachol.
Carbamoylcholine chloride agonist was diluted and added to Muscarinic Acetylcholine Receptor (mAChR) M3/NFAT Luciferase Reporter HEK293 cells for about 5 hours at 37°C in a cell culture incubator.Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690).

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

Sequence

Human mAChR M3 (CHRM3) sequence (accession number: NM_000740.3)

MTLHNNSTTSPLFPNISSSWIHSPSDAGLPPGTVTHFGSYNVSRAAGNFSSPDGTTDDPLGGHTVWQVVFIAFLTGILALVTIIGNI
LVIVSFKVNKQLKTVNNYFLLSLACADLIIGVISMNLFTTYIIMNRWALGNLACDLWLAIDYVASNASVMNLLVISFDRYFSITRPLTY
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LENSASSDEEDIGSETRAIYSIVLKLPGHSTILNSTKLPSSDNLQVPEEELGMVDLERKADKLQAQKSVDDGGSFPKSFSKLPIQLESA
VDTAKTSDVNSSVGKSTATLPLSFKEATLAKRFALKTRSQITKRKRMSLVKEKKAAQTLSAILLAFIITWTPYNIMVLVNTFCDSCIPK
TFWNLGYWLCYINSTVNPVCYALCNKTFRTTFKMLLLCQCDKKKRRKQQYQQRQSVIFHKRAPEQAL

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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 Reporter– HEK293 Cell Line (PKC/Ca^{2+} Pathway)	79298	2 vials
Muscarinic Acetylcholine Receptor (mAChR) M1/NFAT Luciferase Reporter HEK293 Cell Line	82727	2 vials
Muscarinic Acetylcholine Receptor (mAChR) M5/NFAT Luciferase Reporter HEK293 Cell Line	82729	2 vials

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