**Description**

The Muscarinic Acetylcholine Receptor (mAChR) M5/NFAT Luciferase Reporter HEK293 Cell Line is a HEK293 cell line stably expressing the full-length human muscarinic acetylcholine receptor M5 (CHRM5/HM5/M5; accession number: AF498919) 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).

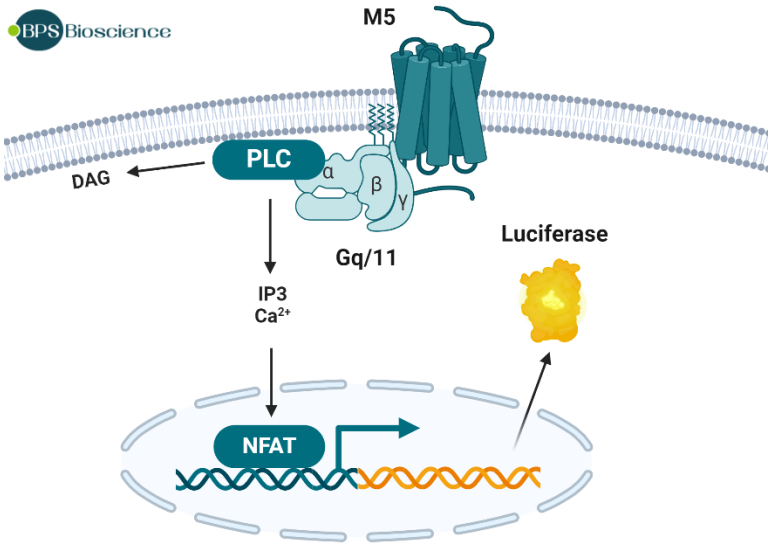


*Figure 1: Diagram illustrating the mechanism of action of Muscarinic Acetylcholine Receptor (mAChR) M5/NFAT Luciferase Reporter HEK293 Cell Line.*
Luciferase activity increases in proportion to mAChR activation.

**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. 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 M5 receptor-related research and drug discovery.
- Counter-screen functionally selective agonists or biased agonists for M1 and M3 receptors.
- Characterize mAChR M5 antibodies/antagonists and agonists in binding assays.

**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 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, 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 *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°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 (#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.

**Cell Culture Protocol**

**Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.**

*Cell Thawing*

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

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

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 1 to the conical tube containing the cells. Thaw Medium 1 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. 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.

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

6. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing culture in a 5% $CO_2$ incubator at 37°C until the cells are ready to be split.

7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1A.

*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 1A 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 1A.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10 to 1:20 weekly 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 1A 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 at least 10 vials at an early passage for future use.*

**Validation**

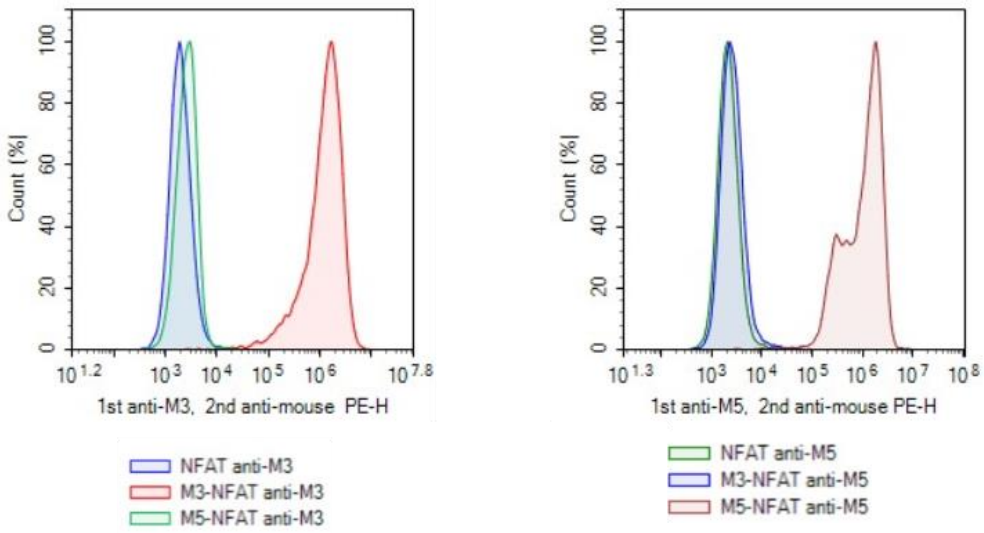


*Figure 2. Flow cytometry analysis of the cell surface expression of human mAChR M3 and mAChR M5 in Muscarinic Acetylcholine Receptor (mAChR) M5/NFAT Luciferase Reporter HEK293 Cell Line.* Muscarinic Acetylcholine Receptor (mAChR) M3/NFAT Luciferase Reporter HEK293 cells (#82728), Muscarinic Acetylcholine Receptor (mAChR) M5/NFAT Luciferase Reporter HEK293 cells and NFAT Reporter HEK293 cells (control; #79298) were stained with Human CHRM3 Antibody (left) or Human CHRM5 Antibody (right), followed by PE Goat anti-mouse IgG (minimal x-reactivity) Antibody, and analyzed by flow cytometry. Y-axis is the % cell number. X-axis is PE intensity.

**Functional Validation**

**A. Muscarinic Acetylcholine Receptor (mAChR) M5/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 “Treated”, “Control” and “Cell-Free Control” conditions.

1. Harvest Muscarinic Acetylcholine Receptor (mAChR) M5/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.

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 concentrations.

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

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

6. Add 100 µl Thaw Medium 1 to "Cell-Free Control" 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 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.

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

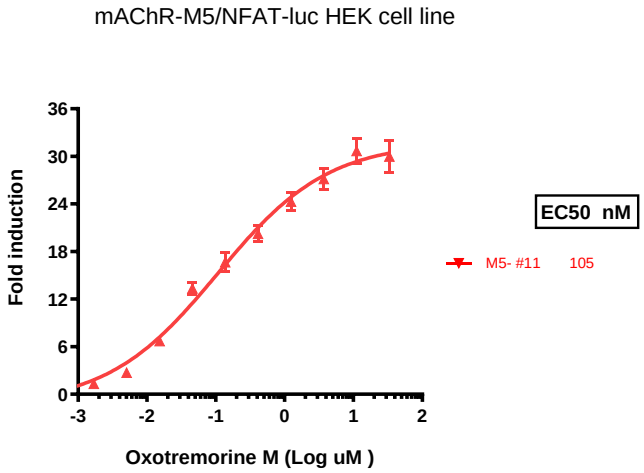


*Figure 3. Dose response of Muscarinic Acetylcholine Receptor (mAChR) M5/NFAT Luciferase Reporter HEK293 Cell Line to Oxotremorine M.*
Oxotremorine M agonist was diluted and added to Muscarinic Acetylcholine Receptor (mAChR) M5/NFAT Luciferase Reporter HEK293 cells for about 5 hours at 37°C in a cell culture incubator. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690).

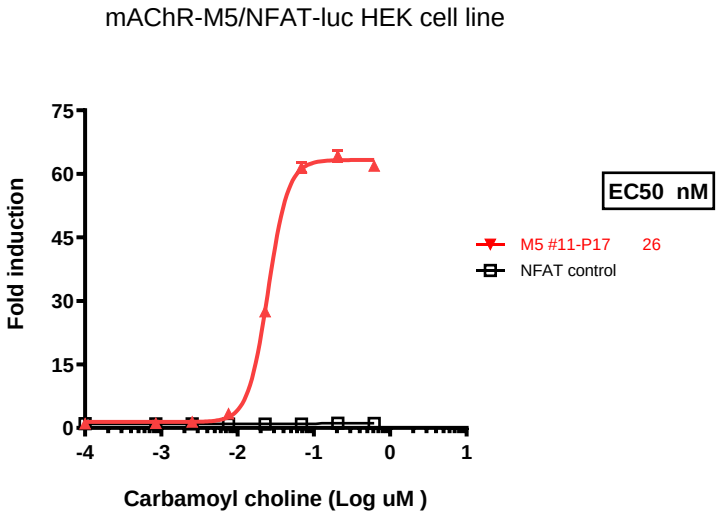


*Figure 4. Dose response of Muscarinic Acetylcholine Receptor (mAChR) M5/NFAT Luciferase Reporter HEK293 Cell Line to carbachol.*
Carbamoylcholine chloride agonist was diluted and added to Muscarinic Acetylcholine Receptor (mAChR) M5/NFAT Luciferase Reporter HEK293 cells for about 5 hours at 37°C in a cell culture incubator. After the treatment, luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690).

*Data shown is representative.*

**Sequence**

Human mAChR M5 (CHRM5) sequence (accession number: AF498919)

MEGDSYHNATTVNGTPVNHQPLERHRLWEVITIAAVTAVVSLITIVGNVLVMISFKVNSQLKTVNNYYLLSLACADLIIGIFSMNLY
TTYILMGRWALGSLACDLWLALDYVASNASVMNLLVISFDRYFSITRPLTYRAKRTPKRAGIMIGLAWLISFILWAPAILCWQYLV
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VVLVKERKAAQTLSAILLAFIITWTPYNIMVLVSTFCDKCVPVTLWHLGYWLCYVNSTVNPICYALCNRTFRKTFKMLLLCRWKKK
KVEEKLYWQGNSKLP

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**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) M3/NFAT Luciferase Reporter HEK293 Cell Line | 82728 | 2 vials |

*Version 071425*