eurofins | Discovery Services

# PRODUCT DATASHEET

## Ready-to-Assay™ $M_5$ Acetylcholine (Muscarinic) Receptor Frozen Cells

## CATALOG NUMBER: HTS075RTA

**CONTENTS**: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

**STORAGE**: Vials are to be stored in liquid $N_2$. Media Component at 4ºC (-20ºC for prolonged storage).

## BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

The muscarinic acetylcholine receptor family consists of five GPCRs that mediate some of the neurotransmission functions of acetylcholine in the CNS and the periphery. The $M_5$ receptor, along with the $M_1$ and $M_3$ receptors, signal through $G_{q/11}$ and subsequent release of $Ca^{++}$ from the ER (Caulfield and Birdsall, 1998). Although $M_5$ is expressed in the CNS at relatively low levels, it appears to be the only muscarinic acetylcholine receptor expressed in midbrain dopaminergic neurons, and it has been shown to mediate dopamine release from these neurons. The $M_5$ receptor is also expressed in blood vessels in the brain and the periphery, and studies with $M_5$ knockout mice demonstrate that $M_5$ mediates the vasodilatory action of acetylcholine on cerebral microvessels. Further studies with $M_5$ knockout mice also indicate a role for $M_5$ in the rewarding effects of cocaine and morphine (Wess, 2004). Cloned human $M_5$-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant $M_5$ expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at $M_5$.

## USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

## WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

### GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

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## APPLICATIONS

Calcium Flux Assays

## APPLICATION DATA

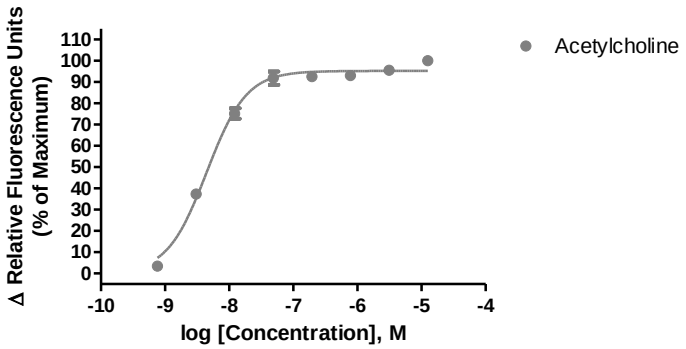


Figure 1. Representative data for activation of $M_5$ receptor. Calcium flux in $M_5$–expressing Chem-1 cell line induced by Acetylcholine. $M_5$–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand, 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR$^{TETRA}$ with ICCD camera. Maximal fluorescence signal obtained in this experiment was 25,000 RLU (Relative Light Units).

Table 1. $EC_{50}$ value of $M_5$-expressing Chem-1 cells.

| LIGAND | ASSAY | POTENCY (nM) | REFERENCE |
|---|---|---|---|
| **Acetylcholine** | Calcium Flux | 4 | Eurofins Internal Data |

## ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.

10. Prepare Fluo-8, AM (AAT Bioquest: 21080) $Ca^{2+}$ dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW $Ca^{2+}$ dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate ($Ca^{2+}$ dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm ($FLIPR^{TETRA}$) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm ($FLIPR^{TETRA}$) or emission filter for $Ca^{2+}$ dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

## ASSAY MATERIALS

| Description | Supplier and Product Number |
|---|---|
| HBSS | Hyclone: SH30268.02 |
| HEPES 1M Stock | EMD Millipore.: TMS-003-C |
| Probenicid | Sigma: P8761 |
| Quest Fluo-8™, AM | AAT Bioquest: 21080 |
| Acetylcholine ligand | Sigma: A6625 |
| Non-binding white plates (for ligand prep) | Corning: 3605(96-well)/3574(384-well) |
| Black (clear bottom) tissue-culture treated plates | Corning: 3904(96-well)/3712(384-well) |

## FLIPR SETTINGS

Settings for $FLIPR^{TETRA}$® with ICCD camera option

| Option | Setting |
|---|---|
| Read Mode | Fluorescence |
| Ex/Em | Ex470_495 / Em515_575 |
| Camera Gain | 2000 |
| Gate Open | 6 % |
| Exposure Time | 0.53 |
| Read Interval | 1s |
| Dispense Volume | 50 µl (25 µl for 384-well) |
| Dispense Height | 25 µl (50 µl for 384-well) |
| Dispense Speed | 75 µl L/sec (50 µl for 384-well) |
| Expel Volume | 0 µl |
| Analysis | Subtract Bias Sample 1 |

# HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

# EXONGENOUS GENE EXPRESSION

$M_5$ cDNA (Accession Number: NM_012125; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

## CODING SEQUENCE

```
                                        ATG GAA GGG GAT TCT TAC CAC AAT GCA ACC ACC GTC AGT GGC ACC
                                         M   E   G   D   S   Y   H   N   A   T   T   V   S   G   T
CCA GTA AAT CAC CAG CCT TTG GAA CGC CAC AGG TTG TGG GAA GTC ATC ACC ATT GCA GCT GTG ACT GCT
 P   V   N   H   Q   P   L   E   R   H   R   L   W   E   V   I   T   I   A   A   V   T   A
GTG GTA AGC CTG ATC ACC ATT GTG GGC AAT GTC TTG GTC ATG ATC TCC TTC AAA GTC AAC AGC CAG CTC
 V   V   S   L   I   T   I   V   G   N   V   L   V   M   I   S   F   K   V   N   S   Q   L
AAG ACA GTT AAC AAC TAT TAC CTG CTC AGC TTA GCC TGT GCA GAT CTC ATC ATT GGA ATC TTC TCC ATG
 K   T   V   N   N   Y   Y   L   L   S   L   A   C   A   D   L   I   I   G   I   F   S   M
AAC CTC TAC ACC ACC TAC ATC CTC ATG GGA CGC TGG GCT CTC GGG AGT CTG GCT TGT GAC CTT TGG CTT
 N   L   Y   T   T   Y   I   L   M   G   R   W   A   L   G   S   L   A   C   D   L   W   L
GCA CTG GAC TAC GTG GCC AGC AAC GCT TCT GTC ATG AAC CTT CTG GTG ATC AGT TTT GAC CGT TAC TTT
 A   L   D   Y   V   A   S   N   A   S   V   M   N   L   L   V   I   S   F   D   R   Y   F
TCC ATC ACA AGA CCC TTG ACA TAT CGG GCC AAG CGT ACT CCG AAA AGG GCT GGC ATC ATG ATT GGC TTG
 S   I   T   R   P   L   T   Y   R   A   K   R   T   P   K   R   A   G   I   M   I   G   L
GCC TGG CTG ATC TCC TTC ATC CTC TGG GCC CCA GCA ATC CTC TGC TGG CAG TAC TTG GTT GGG AAG CGG
 A   W   L   I   S   F   I   L   W   A   P   A   I   L   C   W   Q   Y   L   V   G   K   R
ACA GTT CCA CTG GAT GAG TGC CAG ATC CAG TTT CTC TCT GAG CCC ACC ATC ACT TTT GGC ACT GCC ATT
 T   V   P   L   D   E   C   Q   I   Q   F   L   S   E   P   T   I   T   F   G   T   A   I
GCT GCC TTC TAC ATC CCT GTT TCT GTC ATG ACC ATC CTC TAC TGT CGA ATC TAC CGG GAA ACA GAG AAG
 A   A   F   Y   I   P   V   S   V   M   T   I   L   Y   C   R   I   Y   R   E   T   E   K
CGA ACC AAG GAC CTG GCT GAC CTC CAG GGT TCT GAC TCT GTG ACC AAA GCT GAG AAG AGA AAG CCA GCT
 R   T   K   D   L   A   D   L   Q   G   S   D   S   V   T   K   A   E   K   R   K   P   A
CAT AGG GCT CTG TTC AGA TCC TGC TTG CGC TGT CCT CGA CCC ACC CTG GCT CAG CGG GAA AGG AAC CAG
 H   R   A   L   F   R   S   C   L   R   C   P   R   P   T   L   A   R   Q   E   R   N   Q
GCC TCC TGG TCA TCC TCC CGC AGG AGC ACC TCC ACC ACT GGG AAG CCA TCC CAA GCC ACT GGC CCA AGC
 A   S   W   S   S   S   R   R   S   T   S   T   T   G   K   P   S   Q   A   T   G   P   S
GCC AAT TGG GCC AAA GCT GAG CAG CTC ACC ACC TGT AGC AGC TAC CCT TCC TCA GAG GAT GAG GAC AAG
 A   N   W   A   K   A   E   Q   L   T   T   C   S   S   Y   P   S   S   E   D   E   D   K
CCC GCC ACT GAC CCT GTC CTC CAA GTG GTC TAC AAG AGT CAG GGT AAG GAA AGC CCA GGG GAA GAA TTC
 P   A   T   D   P   V   L   Q   V   V   Y   K   S   Q   G   K   E   S   P   G   E   E   F
AGT GCT GAA GAG ACT GAG GAA ATT TTT GTG AAA GCT GAA ACT GAA AAA AGT GAC TAT GAC ACC CCA AAC
```

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 S   A   E   E   T   E   E   I   F   V   K   A   E   T   E   K   S   D   Y   D   T   P   N

TAC CTT CTG TCT CCA GCA GCT GCT CAT AGA CCC AAG AGT CAG AAA TGT GTG GCC TAT AAG TTC CGA TTG

 Y   L   L   S   P   A   A   A   H   R   P   K   S   Q   K   C   V   A   Y   K   F   R   L

GTG GTA AAA GCT GAC GGG AAC CAG GAG ACC AAC AAT GGC TGT CAC AAG GTG AAA ATC ATG CCC TGC CCC

 V   V   K   A   D   G   N   Q   E   T   N   N   G   C   H   K   V   K   I   M   P   C   P

TTC CCA GTG GCC AAG GAA CCT TCA ACG AAA GGC CTC AAT CCC AAC CCC AGC CAT CAA ATG ACC AAA CGA

 F   P   V   A   K   E   P   S   T   K   G   L   N   P   N   P   S   H   Q   M   T   K   R

AAG AGA GTG GTC CTA GTC AAA GAG AGG AAA GCA GCC CAG ACA CTG AGT GCC ATT CTC CTG GCC TTC ATC

 K   R   V   V   L   V   K   E   R   K   A   A   Q   T   L   S   A   I   L   L   A   F   I

ATC ACA TGG ACC CCG TAT AAC ATC ATG GTC CTG GTT TCT ACC TTC TGT GAC AAG TGT GTC CCA GTC ACC

 I   T   W   T   P   Y   N   I   M   V   L   V   S   T   F   C   D   K   C   V   P   V   T

CTG TGG CAC TTG GGC TAT TGG TTG TGC TAT GTC AAT AGC ACT GTC AAC CCC ATC TGC TAT GCC CTC TGC

 L   W   H   L   G   Y   W   L   C   Y   V   N   S   T   V   N   P   I   C   Y   A   L   C

AAC AGA ACC TTC AGG AAG ACC TTT AAG ATG CTG CTT CTC TGC CGA TGG AAA AAG AAA AAA GTG GAA GAG

 N   R   T   F   R   K   T   F   K   M   L   L   L   C   R   W   K   K   K   K   V   E   E

AAG TTG TAC TGG CAG GGG AAC AGC AAG CTA CCC TGA

K   L   Y   W   Q   G   N   S   K   L   P  Stp
```

## RELATED PRODUCTS

| PRODUCT NUMBER | DESCRIPTION |
|---|---|
| **HTSCHEM-1RTA** | Ready-to-Assay™ Chem-1 host frozen cells (control cells) |
| **HTS075M** | ChemiScreen™ $M_5$ Muscarinic receptor membrane prep |

## REFERENCES


1. Caulfield MP and Birdsall NJM (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.* 50: 279-290.
2. Levey AI (1993). Immunological localization of M1-M5 muscarinic acetylcholine receptors in peripheral tissue and brain. *Life Sci.* 52: 441-448.
3. Wess J. (2004) Muscarinic acetylcholine knockout mice: novel phenotypes and clinical implications. *Annu. Rev. Pharmacol. Toxicol.* 44: 423-450.

## User Agreement (Label License)

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