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# PRODUCT DATASHEET

## Ready-to-Assay™ $M_4$ Acetylcholine (Muscarinic) Receptor Frozen Cells

## CATALOG NUMBER: HTS117RTA

**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 (mAChR) family consists of five GPCRs that mediate some of the neurotransmission functions of acetylcholine in the CNS and the periphery. The $M_1$, $M_3$ and $M_5$ receptors couple to $G_q$ to mobilize intracellular calcium, whereas the $M_2$ and $M_4$ receptors couple to $G_{i/o}$ to inhibit cAMP production (Caulfield and Birdsall, 1998. Muscarinic $M_4$ receptor is known to be abundantly expressed in the striatum (Levey 1993). Consistent with this fact, locomotor activity was significantly increased in $M_4$ receptor-deficient mice (Gomeza *et al.* 1999). Cloned human $M_4$-expressing cell line is made in the Chem-4 host, which supports high levels of recombinant $M_4$ expression on the cell surface and contains optimized levels of a recombinant promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at $M_4$.

## 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.

**Eurofins Pharma Bioanalytics Services US Inc.**
15 Research Park Drive
St Charles MO 63304
USA
T +1 844 522 7787
F +1 636 362 7131
www.eurofins.com

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## APPLICATIONS

Calcium Flux Assays

## APPLICATION DATA

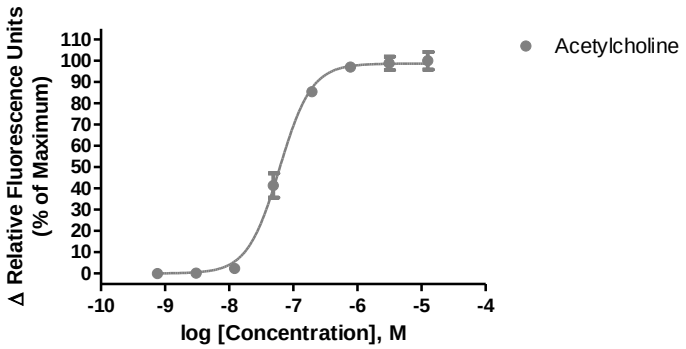


Figure 1. Representative data for activation of $M_4$ receptor. Calcium flux in $M_4$–expressing Chem-4 cell line induced by Acetylcholine. $M_4$–expressing Chem-4 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand, 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR$^{TETRA}$ with ICCD camera. Maximal fluorescence signal obtained in this experiment was 15,000 RLU (Relative Light Units).

Table 1. $EC_{50}$ values of $M_4$-expressing Chem-4 cells.

| LIGAND | ASSAY | POTENCY (nM) | REFERENCE |
|---|---|---|---|
| **Acetylcholine** | Calcium Flux | 60 | 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: D8040 |
| 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-4, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein as well as an exogenous proprietary promiscuous Gα protein.

## EXONGENOUS GENE EXPRESSION

$M_4$ cDNA (Accession Number: NM_00741; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

## CODING SEQUENCE

```
ATG GCC AAC TTC ACA CCT GTC AAT GGC AGC TCG GGC AAT CAG TCC GTG CGC CTG GTC ACG TCA TCA TCC      69
 M   A   N   F   T   P   V   N   G   S   S   G   N   Q   S   V   R   L   V   T   S   S   S      23

CAC AAT CGC TAT GAG ACG GTG GAA ATG GTC TTC ATT GCC ACA GTG ACA GGC TCC CTG AGC CTG GTG ACT     138
 H   N   R   Y   E   T   V   E   M   V   F   I   A   T   V   T   G   S   L   S   L   V   T      46

GTC GTG GGC AAC ATC CTG GTG ATG CTG TCC ATC AAG GTC AAC AGG CAG CTG CAG ACA GTC AAC AAC TAC     207
 V   V   G   N   I   L   V   M   L   S   I   K   V   N   R   Q   L   Q   T   V   N   N   Y      69

TTC CTC TTC AGC CTG GCG TGT GCT GAT CTC ATC ATA GGC GCC TTC TCC ATG AAC CTC TAC ACC GTG TAC     276
 F   L   F   S   L   A   C   A   D   L   I   I   G   A   F   S   M   N   L   Y   T   V   Y      92

ATC ATC AAG GGC TAC TGG CCC CTG GGC GCC GTG GTC TGC GAC CTG TGG CTG GCC CTG GAC TAC GTG GTG     345
 I   I   K   G   Y   W   P   L   G   A   V   V   C   D   L   W   L   A   L   D   Y   V   V     115

AGC AAC GCC TCC GTC ATG AAC CTT CTC ATC ATC AGC TTT GGC CGC TAC TTC TGC GTC ACC AAG CCT CTC     414
 S   N   A   S   V   M   N   L   L   I   I   S   F   G   R   Y   F   C   V   T   K   P   L     138

ACC TAC CCT GCC CGG CGC ACC ACC AAG ATG GCA GGC CTC ATG ATT GCT GCT GCC TGG GTA CTG TCC TTC     483
 T   Y   P   A   R   R   T   T   K   M   A   G   L   M   I   A   A   A   W   V   L   S   F     161

GTG CTC TGG GCG CCT GCC ATC TTG TTC TGG CAG TTT GTG GTG GGT AAG CGG ACG GTG CCC GAC AAC CAG     552
 V   L   W   A   P   A   I   L   F   W   Q   F   V   V   G   K   R   T   V   P   D   N   Q     184

TGC TTC ATC CAG TTC CTG TCC AAC CCA GCA GTG ACC TTT GGC ACG GCC ATT GCT GCC TTC TAC CTG CCT     621
 C   F   I   Q   F   L   S   N   P   A   V   T   F   G   T   A   I   A   A   F   Y   L   P     207

GTG GTC ATC ATG ACG GTG CTG TAC ATC CAC ATC TCC CTG GCC AGT CGC AGC CGA GTC CAC AAG CAC CGG     690
 V   V   I   M   T   V   L   Y   I   H   I   S   L   A   S   R   S   R   V   H   K   H   R     230

CCC GAG GGC CCG AAG GAG AAG AAA GCC AAG ACG CTG GCC TTC CTC AAG AGC CCA CTA ATG AAG CAG AGC     759
 P   E   G   P   K   E   K   K   A   K   T   L   A   F   L   K   S   P   L   M   K   Q   S     253

GTC AAG AAG CCC CCG CCC GGG GAG GCC GCC CGG GAG GAG CTG CGC AAT GGC AAG CTG GAG GAG GCC CCC     828
 V   K   K   P   P   P   G   E   A   A   R   E   E   L   R   N   G   K   L   E   E   A   P     276

CCG CCA GCG CTG CCA CCG CCA CCG CGC CCC GTG GCT GAT AAG GAC ACT TCC AAT GAG TCC AGC TCA GGC     897
 P   P   A   L   P   P   P   P   R   P   V   A   D   K   D   T   S   N   E   S   S   S   G     299

AGT GCC ACC CAG AAC ACC AAG GAA CGC CCA GCC ACA GAG CTG TCC ACC ACA GAG GCC ACC ACG CCC GCC     966
 S   A   T   Q   N   T   K   E   R   P   A   T   E   L   S   T   T   E   A   T   T   P   A     322

ATG CCC GCC CCT CCC CTG CAG CCG CGG GCC CTC AAC CCA GCC TCC AGA TGG TCC AAG ATC CAG ATT GTG    1035
 M   P   A   P   P   L   Q   P   R   A   L   N   P   A   S   R   W   S   K   I   Q   I   V     345

ACG AAG CAG ACA GGC AAT GAG TGT GTG ACA GCC ATT GAG ATT GTG CCT GCC ACG CCG GCT GGC ATG CGC    1104
 T   K   Q   T   G   N   E   C   V   T   A   I   E   I   V   P   A   T   P   A   G   M   R     368

CCT GCG GCC AAC GTG GCC CGC AAG TTC GCC AGC ATC GCT CGC AAC CAG GTG CGC AAG AAG CGG CAG ATG    1173
 P   A   A   N   V   A   R   K   F   A   S   I   A   R   N   Q   V   R   K   K   R   Q   M     391

GCG GCC CGG GAG CGC AAA GTG ACA CGA ACG ATC TTT GCC ATT CTG CTA GCC TTC ATC CTC ACC TGG ACG    1242
 A   A   R   E   R   K   V   T   R   T   I   F   A   I   L   L   A   F   I   L   T   W   T     414

CCC TAC AAC GTC ATG GTC CTG GTG AAC ACC TTC TGC CAG AGC TGC ATC CCT GAC ACG GTG TGG TCC ATT    1311
 P   Y   N   V   M   V   L   V   N   T   F   C   Q   S   C   I   P   D   T   V   W   S   I     437

GGC TAC TGG CTC TGC TAC GTC AAC AGC ACT ATC AAC CCT GCC TGC TAT GCT CTG TGC AAC GCC ACC TTT    1380
 G   Y   W   L   C   Y   V   N   S   T   I   N   P   A   C   Y   A   L   C   N   A   T   F     460

AAA AAG ACC TTC CGG CAC CTG CTG CTG TGC CAG TAT CGG AAC ATC GGC ACT GCC AGG TGA               1443
 K   K   T   F   R   H   L   L   L   C   Q   Y   R   N   I   G   T   A   R Stp               479
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## RELATED PRODUCTS

| PRODUCT NUMBER | DESCRIPTION |
|---|---|
| **HTSCHEM-1RTA** | Ready-to-Assay™ Chem-1 host frozen cells (control cells) |

* Note: Chem-4 cells are derived from Chem-1 cells

## 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. Gomeza J *et al*. (1999) Enhancement of D1 dopamine receptor-mediated locomotor stimulation in M4 muscarinic acetylcholine receptor knockout mice. *Proc. Natl. Acad. Sci. USA* 96: 10483-10488.


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