eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ M_2 Acetylcholine (Muscarinic) Family Receptor Frozen Cells

CATALOG NUMBER: HTS115RTA

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 over night 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 Gq to mobilize intracellular calcium, whereas the M_2 and M_4 receptors couple to Gi/o to inhibit cAMP production (Caulfield and Birdsall, 1998). In urinary bladder trachea and stomach, M_2 augments the function of M_3 in promoting contractility, and activation of M_2 serves to counteract relaxation induced by increased cAMP levels (Ehlert et al., 2005; Wess, 2004). In addition, the ability of mChR agonists to decrease heart rate appears to be mediated primarily by M_2. Agonists of mAChRs induce tremor, hypothermia, corticosterone release, and analgesia; each of these functions is mediated at least in part by M_2 (Wess, 2004). Cloned human M_2 -expressing cell line is made in the Chem-1 host, which supports high levels of recombinant M_2 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_2.

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.

APPLICATIONS

Calcium Flux Assays

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T | +1 844 522 7787
F | +1 636 441 6058
www.eurofins.com

APPLICATION DATA

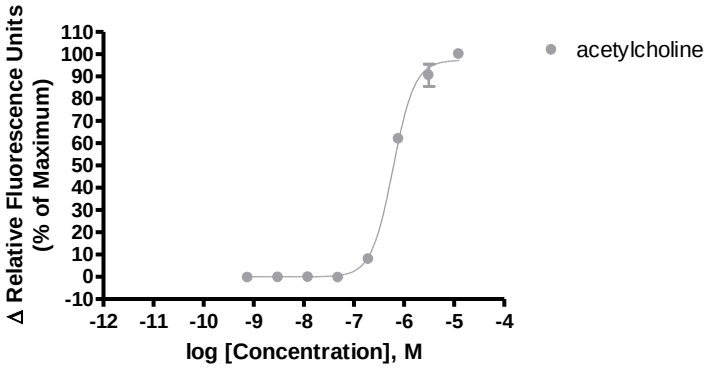

Figure 1. Representative data for activation of M_2 receptor. Calcium flux in M_2 –expressing Chem-1 cell line induced by acetylcholine. M_2 –expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 3,300 RLU (Relative Light Units).

Table I. Comparison of EC_{50} values of M_2 -expressing Chem-1 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Acetylcholine	Calcium Flux	600	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: SH3026802
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
U-50488 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-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein

EXONGENOUS GENE EXPRESSION

CHRM2 cDNA (Accession Number: NM_000739; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

```
                                                ATG AAT AAC TCA ACA AAC TCC TCT AAC AAT AGC CTG
                                                  M   N   N   S   T   N   S   S   N   N   S   L

GCT CTT ACA AGT CCT TAT AAG ACA TTT GAA GTG GTG TTT ATT GTC CTG GTG GCT GGA TCC CTC AGT TTG
 A   L   T   S   P   Y   K   T   F   E   V   V   F   I   V   L   V   A   G   S   L   S   L

GTG ACC ATT ATC GGG AAC ATC CTA GTC ATG GTT TCC ATT AAA GTC AAC CGC CAC CTC CAG ACC GTC AAC
 V   T   I   I   G   N   I   L   V   M   V   S   I   K   V   N   R   H   L   Q   T   V   N

AAT TAC TTT TTA TTC AGC TTG GCC TGT GCT GAC CTT ATC ATA GGT GTT TTC TCC ATG AAC TTG TAC ACC
 N   Y   F   L   F   S   L   A   C   A   D   L   I   I   G   V   F   S   M   N   L   Y   T

CTC TAC ACT GTG ATT GGT TAC TGG CCT TTG GGA CCT GTG GTG TGT GAC CTT TGG CTA GCC CTG GAC TAT
 L   Y   T   V   I   G   Y   W   P   L   G   P   V   V   C   D   L   W   L   A   L   D   Y

GTG GTC AGC AAT GCC TCA GTT ATG AAT CTG CTC ATC ATC AGC TTT GAC AGG TAC TTC TGT GTC ACA AAA
 V   V   S   N   A   S   V   M   N   L   L   I   I   S   F   D   R   Y   F   C   V   T   K

CCT CTG ACC TAC CCA GTC AAG CGG ACC ACA AAA ATG GCA GGT ATG ATG ATT GCA GCT GCC TGG GTC CTC
 P   L   T   Y   P   V   K   R   T   T   K   M   A   G   M   M   I   A   A   A   W   V   L

TCT TTC ATC CTC TGG GCT CCA GCC ATT CTC TTC TGG CAG TTC ATT GTA GGG GTG AGA ACT GTG GAG GAT
 S   F   I   L   W   A   P   A   I   L   F   W   Q   F   I   V   G   V   R   T   V   E   D

GGG GAG TGC TAC ATT CAG TTT TTT TCC AAT GCT GCT GTC ACC TTT GGT ACG GCT ATT GCA GCC TTC TAT
 G   E   C   Y   I   Q   F   F   S   N   A   A   V   T   F   G   T   A   I   A   A   F   Y

TTG CCA GTG ATC ATC ATG ACT GTG CTA TAT TGG CAC ATA TCC CGA GCC AGC AAG AGC AGG ATA AAG AAG
 L   P   V   I   I   M   T   V   L   Y   W   H   I   S   R   A   S   K   S   R   I   K   K

GAC AAG AAG GAG CCT GTT GCC AAC CAA GAC CCC GTT TCT CCA AGT CTG GTA CAA GGA AGG ATA GTG AAG
 D   K   K   E   P   V   A   N   Q   D   P   V   S   P   S   L   V   Q   G   R   I   V   K

CCA AAC AAT AAC AAC ATG CCC AGC AGT GAC GAT GGC CTG GAG CAC AAC AAA ATC CAG AAT GGC AAA GCC
 P   N   N   N   N   M   P   S   S   D   D   G   L   E   H   N   K   I   Q   N   G   K   A

CCC AGG GAT CCT GTG ACT GAA AAC TGT GTT CAG GGA GAG GAG AAG GAG AGC TCC AAT GAC TCC ACC TCA
 P   R   D   P   V   T   E   N   C   V   Q   G   E   E   K   E   S   S   N   D   S   T   S

GTC AGT GCT GTT GCC TCT AAT ATG AGA GAT GAT GAA ATA ACC CAG GAT GAA AAC ACA GTT TCC ACT TCC
 V   S   A   V   A   S   N   M   R   D   D   E   I   T   Q   D   E   N   T   V   S   T   S

CTG GGC CAT TCC AAA GAT GAG AAC TCT AAG CAA ACA TGC ATC AGA ATT GGC ACC AAG ACC CCA AAA AGT
 L   G   H   S   K   D   E   N   S   K   Q   T   C   I   R   I   G   T   K   T   P   K   S

GAC TCA TGT ACC CCA ACT AAT ACC ACC GTG GAG GTA GTG GGG TCT TCA GGT CAG AAT GGA GAT GAA AAG
 D   S   C   T   P   T   N   T   T   V   E   V   V   G   S   S   G   Q   N   G   D   E   K

CAG AAT ATT GTA GCC CGC AAG ATT GTG AAG ATG ACT AAG CAG CCT GCA AAA AAG AAG CCT CCT CCT TCC
 Q   N   I   V   A   R   K   I   V   K   M   T   K   Q   P   A   K   K   K   P   P   P   S

CGG GAA AAG AAA GTC ACC AGG ACA ATC TTG GCT ATT CTG TTG GCT TTC ATC ATC ACT TGG GCC CCA TAC
 R   E   K   K   V   T   R   T   I   L   A   I   L   L   A   F   I   I   T   W   A   P   Y

AAT GTC ATG GTG CTC ATT AAC ACC TTT TGT GCA CCT TGC ATC CCC AAC ACT GTG TGG ACA ATT GGT TAC
 N   V   M   V   L   I   N   T   F   C   A   P   C   I   P   N   T   V   W   T   I   G   Y

TGG CTT TGT TAC ATC AAC AGC ACT ATC AAC CCT GCC TGC TAT GCA CTT TGC AAT GCC ACC TTC AAG AAG
 W   L   C   Y   I   N   S   T   I   N   P   A   C   Y   A   L   C   N   A   T   F   K   K

ACC TTT AAA CAC CTT CTC ATG TGT CAT TAT AAG AAC ATA GGC GCT ACA AGG TGA
 T   F   K   H   L   L   M   C   H   Y   K   N   I   G   A   T   R  Stp
```

RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS115M	ChemiScreen™ M_2 acetylcholine (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. Ehlert FJ *et al.* (2005) The M_2 muscarinic receptor mediates contraction through indirect mechanisms in mouse urinary bladder. *J. Pharmacol. Exp. Ther.* 313: 368-378.
3. Wess J (2004) Muscarinic acetylcholine knockout mice: novel phenotypes and clinical implications. *Annu. Rev. Pharmacol. Toxicol.* 44: 423-450.

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

User Agreement (Label License)

In addition to the General Terms and Conditions section, thee specific terms also apply for **M_2 Acetylcholine (Muscarinic) Family Receptor Frozen Cells, Product No. HTS115RTA**

BY USING THE THIS PRODUCT LICENSED TO YOU ("LICENSEE") HEREUNDER, YOU ARE HEREBY REPRESENTING THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF OR YOUR COMPANY, AS APPLICABLE, AND ARE CONSENTING TO BE LEGALLY BOUND BY ALL OF THE TERMS OF THIS USER AGREEMENT ("AGREEMENT"). IF YOU DO NOT AGREE TO ALL THESE TERMS, DO NOT USE THE PRODUCT, AND IMMEDIATELY RETURN SUCH PRODUCTS TO THE APPLICABLE SELLER FOR A REFUND. This is a legal agreement between Licensee and Eurofins Pharma Bioanalytics Services US Inc. governing use of the Ready-to-Assay Cells products and/or any accompanying operating/use protocols (the "Product(s)") provided to Licensee.

LICENSEE shall obtain no ownership interest in the Product or use/culture protocols accompanying the Product other than through the perpetual limited license granted herein. If the Product is licensed through an authorized Eurofins Pharma Bioanalytics Services US Inc. distributor, Licensee shall be obligated to disclose its identity to Eurofins Pharma Bioanalytics Services US Inc. to insure compliance with this User Agreement.

Limited License and Restrictions. Pursuant to the terms and conditions of this Agreement, Eurofins Pharma Bioanalytics Services US Inc. conveys to Licensee the non-exclusive and non-transferable right to use the Licensed Product only for Research Purposes conducted by Licensee (whether Licensee is an academic user or a for-profit entity). "Research Purposes" means any biological research and development application or use, including without limitation, developing, demonstrating or validating biological assays, life sciences and/or pharmaceutical research. "Research Purposes" excludes applications outside biology (including but not limited to consumer electronics or materials sciences), and specifically excludes the following applications of whatever kind or nature: Clinical Diagnostics (any use of a product or service for clinical diagnosis where data from an individual's sample is given to such individual or used for the purpose of diagnosis or treatment of a medical condition in such individual, where that result may be used in the treatment of such individual), therapeutics, clinical imaging, environmental testing and cosmetics. Contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee. Licensee cannot sell or otherwise transfer (a) this Product or (b) materials made using this Product to a third party. Licensee may transfer information or materials made through use of this Product to a scientific collaborator, provided that such transfer is not for the commercial purposes, and that such collaborator agrees in writing: (a) not to

transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for Research Purposes and not for commercial purposes. Commercial purposes means any activity by a user of the Product for consideration that may include, but is not limited to: (1) operating a service business that uses the Products to develop information or data which is resold for research and development applications; (2) use of the Product in manufacturing; (3) use of the Product for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the Product, whether or not such Product is resold for use in research. Licensee expressly represents and warrants to Eurofins Pharma Bioanalytics Services US Inc. that Licensee will properly test and use any Product purchased from Eurofins Pharma Bioanalytics Services US Inc. or its affiliated companies in accordance with the practices of a reasonable person who is an expert in the field and in strict compliance with all applicable laws and regulations, now and hereinafter enacted. Licensee agrees to comply with instructions, if any, furnished by Eurofins Pharma Bioanalytics Services US Inc. relating to the use of the Product and to not misuse the Product in any manner. Licensee shall not reverse engineer, disassemble or modify the Product or create any derivative works of the written materials accompanying the Product, including but not limited to any material data sheets or similar materials with respect to the Products' specifications. Licensee acknowledges that Eurofins Pharma Bioanalytics Services US Inc. or its affiliated companies retains ownership of all patents, copyrights, trademarks, trade secrets and other proprietary rights relating to or residing in the Product or any portion thereof.

Term and Termination. This Agreement commences upon Licensee's use of the Products, and shall remain in effect in perpetuity unless terminated sooner as set forth hereunder. Eurofins Pharma Bioanalytics Services US Inc. may terminate this Agreement immediately if Licensee breaches any provision herein. Upon any such termination, all rights granted to Licensee hereunder will immediately terminate, and Licensee shall immediately cease using the Product and, at Eurofins Pharma Bioanalytics Services US Inc.'s option, return or destroy all Products (certifying such destruction to Eurofins Pharma Bioanalytics Services US Inc. in writing).

Assignment. Licensee shall not sublicense, assign (by operation of law of otherwise) or otherwise transfer this Agreement or any of the rights or licenses granted under this Agreement without the prior written consent of Eurofins Pharma Bioanalytics Services US Inc.. Any attempted assignment, sublicense or transfer by Licensee without such consent shall be null and void.