

PRODUCT DATASHEET

Ready-to-Assay™ M₁ Acetylcholine (Muscarinic) Family Receptor Frozen Cells

CATALOG NUMBER: HTS044RTA

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 N₂. Media Component at 4°C (-20°C 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 M₁ receptor, along with the M₃ and M₅ receptors, signal through Gq/11 and subsequent release of Ca⁺⁺ from the ER. The M₁ receptor is expressed in ganglia and mediates depolarization of ganglia by inhibition of voltage-gated M-type K⁺ channels. In addition, the M₁ receptor mediates venous contraction (Caulfield and Birdsall, 1998). Cloned human M₁-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant M₁ 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 M₁.

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

APPLICATION DATA

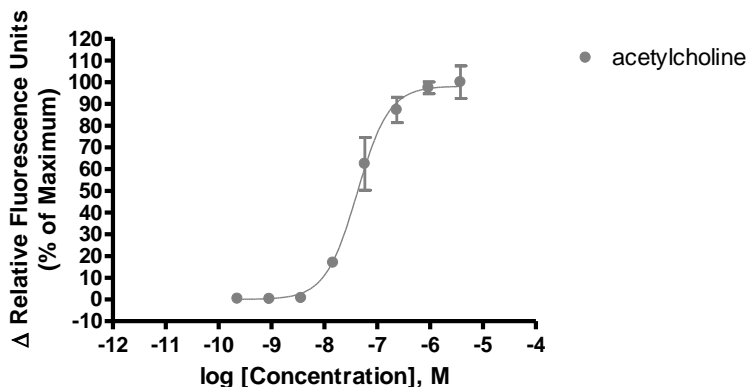


Figure 1. Representative data for activation of M₁ receptor. Calcium flux in M₁-expressing Chem-1 cell line induced by acetylcholine. M₁-expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 2,600 RLU (Relative Light Units).

Table 1. EC₅₀ value of M₁-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
acetylcholine	Calcium Flux	40	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 37°C 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 37°C 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) Ca²⁺ dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca²⁺ dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca²⁺ 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 Ca²⁺ 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
Probenecid	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

CHRM1 cDNA (Accession Number: NM_000738; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

```

ATG AAC ACT TCA GCC CCA CCT GCT GTC AGC CCC AAC ATC ACC GTC CTG GCA CCA GGA AAG GGT CCC TGG CAA GTG GCC TTC
ATT GGG ATC ACC ACG GGC CTC CTG TCG CTA GCC ACA GTG ACA GGC AAC CTG CTG GTA CTC ATC TCT TTC AAG GTC AAC ACG
GAG CTC AAG ACA GTC AAT AAC TAC TTC CTG CTG AGC CTG GCC TGT GCT GAC CTC ATC ATC GGT ACC TTC TCC ATG AAC CTC
TAT ACC ACG TAC CTG CTC ATG GGC CAC TGG GCT CTG GGC ACG CTG GCT TGT GAC CTC TGG CTG GCC CTG GAC TAT GTG GCC
AGC AAT GCC TCC GTC ATG AAT CTG CTG CTC ATC AGC TTT GAC CGC TAC TTC TCC GTG ACT CGG CCC CTG AGC TAC CGT GCC
AAG CGC ACA CCC CGC CGG GCA GCT CTG ATG ATC GGC CTG GCC TGG CTG GTT TCC TTT GTG CTC TGG GCC CCA GCC ATC CTC
TTC TGG CAG TAC CTG GTA GGG GAG CGG ACA GTG CTA GCT GGG CAG TGC TAC ATC CAG TTC CTC TCC CAG CCC ATC ATC ACC
TTT GGC ACA GCC ATG GCT GCC TTC TAC CTC CCT GTC ACA GTC ATG TGC ACG CTC TAC TGG CGC ATC TAC CGG GAG ACA GAG
AAC CGA GCA CGG GAG CTG GCA GCC CTT CAG GGC TCC GAG ACG CCA GGC AAA GGG GGT GGC AGC AGC AGC AGC TCA GAG AGG
TCT CAG CCA GGG GCT GAG GGC TCA CCA GAG ACT CCT CCA GGC CGC TGC TGT CGC TGC TGC CGG GCC CCC AGG CTG CTG CAG
GCC TAC AGC TGG AAG GAA GAA GAG GAA GAG GAC GAA GGC TCC ATG GAG TCC CTC ACA TCC TCA GAG GGA GAG GAG CCT GGC
TCC GAA GTG GTG ATC AAG ATG CCA ATG GTG GAC CCC GAG GCA CAG GCC CCC ACC AAG CAG CCC CCA CGG AGC TCC CCA AAT
ACA GTC AAG AGG CCG ACT AAG AAA GGG CGT GAT CGA GCT GGC AAG GGC CAG AAG CCC CGT GGA AAG GAG CAG CTG GCC AAG
CGG AAG ACC TTC TCG CTG GTC AAG GAG AAG AAG GCG GCT CGG ACC CTG AGT GCC ATC CTC CTG GCC TTC ATC CTC ACC TGG
ACA CCG TAC AAC ATC ATG GTG CTG GTG TCC ACC TTC TGC AAG GAC TGT GTT CCC GAG ACC CTG TGG GAG CTG GGC TAC TGG
CTG TGC TAC GTC AAC AGC ACC ATC AAC CCC ATG TGC TAC GCA CTC TGC AAC AAA GCC TTC CGG GAC ACC TTT CGC CTG CTG
CTG CTT TGC CGC TGG GAC AAG AGA CGC TGG CGC AAG ATC CCC AAG CGC CCT GGC TCC GTG CAC CGC ACT CCC TCC CGC CAA
TGC TGA
    
```

RELATED PRODUCTS

PRODUCT NUMBER

DESCRIPTION

HTSCHEM-1RTA

Ready-to-Assay™ Chem-1 host frozen cells (control cells)

HTS044M

ChemiScreen™ M₁ acetylcholine (muscarinic) family receptor membrane prep

REFERENCES

1. Caulfield M.P. and Birdsall N.J.M. (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.* 50: 279-290.

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

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

No part of these works may be reproduced in any form without permission in writing.

User Agreement (Label License)

In addition to the General Terms and Conditions section, these specific terms also apply for **Ready-to-Assay™ M₁ Acetylcholine (Muscarinic) Family Receptor Frozen Cells, Product No. HTS044RTA**

BY USING 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 or 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.