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PRODUCT DATASHEET

Ready-to-Assay™ M_3 Acetylcholine (Muscarinic) Family Receptor Frozen Cells

CATALOG NUMBER: HTS116RTA

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 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). M_3 is expressed prominently in smooth muscle, and plays a primary role in mediating mAChR agonist-induced contractility. Mice lacking M_3 have dilated pupils, which indicate a role for M_3 in regulating tone of the pupillary sphincter muscle. In addition, M_3 plays a role in feeding, as indicated by the lean and hypophagic phenotype of M_3-null mice (Wess, 2004). Cloned human M_3 -expressing cell line is made in the Chem-1 host, which supports high levels of recombinant M_3 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_3.

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

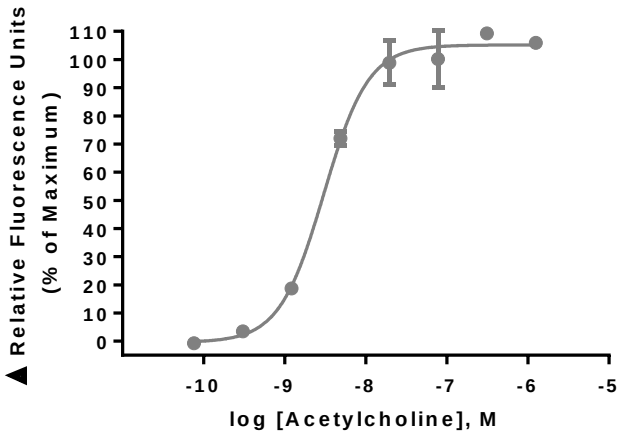

Figure 1. Representative data for activation of M_3 receptor. Calcium flux in M_3 –expressing Chem-1 cell line induced by acetylcholine. M_3 –expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 4,500 RLU (Relative Light Units).

Table 1. EC_{50} values of M_3 -expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50}(nM)	REFERENCE
acetylcholine	Calcium Flux	3.0	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 invert plate to remove 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: C4382
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

CHRM3 cDNA (Accession Number: NM_000740; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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ATG ACC TTG CAC AAT AAC AGT ACA ACC TCG CCT TTG TTT CCA AAC ATC AGC TCC TCC TGG ATA CAC AGC     69
 M   T   L   H   N   N   S   T   T   S   P   L   F   P   N   I   S   S   S   W   I   H   S     23

CCC TCC GAT GCA GGG CTG CCC CCG GGA ACC GTC ACT CAT TTC GGC AGC TAC AAT GTT TCT CGA GCA GCT    138
 P   S   D   A   G   L   P   P   G   T   V   T   H   F   G   S   Y   N   V   S   R   A   A     46

GGC AAT TTC TCC TCT CCA GAC GGT ACC ACC GAT GAC CCT CTG GGA GGT CAT ACC GTC TGG CAA GTG GTC    207
 G   N   F   S   S   P   D   G   T   T   D   D   P   L   G   G   H   T   V   W   Q   V   V     69

TTC ATC GCT TTC TTA ACG GGC ATC CTG GCC TTG GTG ACC ATC ATC GGC AAC ATC CTG GTA ATT GTG TCA    276
 F   I   A   F   L   T   G   I   L   A   L   V   T   I   I   G   N   I   L   V   I   V   S     92

TTT AAG GTC AAC AAG CAG CTG AAG ACG GTC AAC AAC TAC TTC CTC TTA AGC CTG GCC TGT GCC GAT CTG    345
 F   K   V   N   K   Q   L   K   T   V   N   N   Y   F   L   L   S   L   A   C   A   D   L    115

ATT ATC GGG GTC ATT TCA ATG AAT CTG TTT ACG ACC TAC ATC ATC ATG AAT CGA TGG GCC TTA GGG AAC    414
 I   I   G   V   I   S   M   N   L   F   T   T   Y   I   I   M   N   R   W   A   L   G   N    138

TTG GCC TGT GAC CTC TGG CTT GCC ATT GAC TAC GTA GCC AGC AAT GCC TCT GTT ATG AAT CTT CTG GTC    483
 L   A   C   D   L   W   L   A   I   D   Y   V   A   S   N   A   S   V   M   N   L   L   V    161

ATC AGC TTT GAC AGA TAC TTT TCC ATC ACG AGG CCG CTC ACG TAC CGA GCC AAA CGA ACA ACA AAG AGA    552
 I   S   F   D   R   Y   F   S   I   T   R   P   L   T   Y   R   A   K   R   T   T   K   R    184

GCC GGT GTG ATG ATC GGT CTG GCT TGG GTC ATC TCC TTT GTC CTT TGG GCT CCT GCC ATC TTG TTC TGG    621
 A   G   V   M   I   G   L   A   W   V   I   S   F   V   L   W   A   P   A   I   L   F   W    207

CAA TAC TTT GTT GGA AAG AGA ACT GTG CCT CCG GGA GAG TGC TTC ATT CAG TTC CTC AGT GAG CCC ACC    690
 Q   Y   F   V   G   K   R   T   V   P   P   G   E   C   F   I   Q   F   L   S   E   P   T    230

ATT ACT TTT GGC ACA GCC ATC GCT GCT TTT TAT ATG CCT GTC ACC ATT ATG ACT ATT TTA TAC TGG AGG    759
 I   T   F   G   T   A   I   A   A   F   Y   M   P   V   T   I   M   T   I   L   Y   W   R    253

ATC TAT AAG GAA ACT GAA AAG CGT ACC AAA GAG CTT GCT GGC CTG CAA GCC TCT GGG ACA GAG GCA GAG    828
 I   Y   K   E   T   E   K   R   T   K   E   L   A   G   L   Q   A   S   G   T   E   A   E    276

 ACA GAA AAC TTT GTC CAC CCC ACG GGC AGT TCT CGA AGC TGC AGC AGT TAC GAA CTT CAA CAG CAA AGC    897
 T   E   N   F   V   H   P   T   G   S   S   R   S   C   S   S   Y   E   L   Q   Q   Q   S    299

 ATG AAA CGC TCC AAC AGG AGG AAG TAT GGC CGC TGC CAC TTC TGG TTC ACA ACC AAG AGC TGG AAA CCC    966
 M   K   R   S   N   R   R   K   Y   G   R   C   H   F   W   F   T   T   K   S   W   K   P    322

 AGC TCC GAG CAG ATG GAC CAA GAC CAC AGC AGC AGT GAC AGT TGG AAC AAC AAT GAT GCT GCT GCC TCC   1035
 S   S   E   Q   M   D   Q   D   H   S   S   S   D   S   W   N   N   N   D   A   A   A   S    345

CTG GAG AAC TCC GCC TCC TCC GAC GAG GAG GAC ATT GGC TCC GAG ACG AGA GCC ATC TAC TCC ATC GTG   1104
 L   E   N   S   A   S   S   D   E   E   D   I   G   S   E   T   R   A   I   Y   S   I   V    368

CTC AAG CTT CCG GGT CAC AGC ACC ATC CTC AAC TCC ACC AAG TTA CCC TCA TCG GAC AAC CTG CAG GTG   1173
 L   K   L   P   G   H   S   T   I   L   N   S   T   K   L   P   S   S   D   N   L   Q   V    391

CCT GAG GAG GAG CTG GGG ATG GTG GAC TTG GAG AGG AAA GCC GAC AAG CTG CAG GCC CAG AAG AGC GTG   1242
 P   E   E   E   L   G   M   V   D   L   E   R   K   A   D   K   L   Q   A   Q   K   S   V    414

GAC GAT GGA GGC AGT TTT CCA AAA AGC TTC TCC AAG CTT CCC ATC CAG CTA GAG TCA GCC GTG GAC ACA   1311
 D   D   G   G   S   F   P   K   S   F   S   K   L   P   I   Q   L   E   S   A   V   D   T    437

GCT AAG ACT TCT GAC GTC AAC TCC TCA GTG GGT AAG AGC ACG GCC ACT CTA CCT CTG TCC TTC AAG GAA   1380
 A   K   T   S   D   V   N   S   S   V   G   K   S   T   A   T   L   P   L   S   F   K   E    460

GCC ACT CTG GCC AAG AGG TTT GCT CTG AAG ACC AGA AGT CAG ATC ACT AAG CGG AAA AGG ATG TCC CTG   1449
 A   T   L   A   K   R   F   A   L   K   T   R   S   Q   I   T   K   R   K   R   M   S   L    483

GTC AAG GAG AAG AAA GCG GCC CAG ACC CTC AGT GCG ATC TTG CTT GCC TTC ATC ATC ACT TGG ACC CCA   1518
 V   K   E   K   K   A   A   Q   T   L   S   A   I   L   L   A   F   I   I   T   W   T   P    506

TAC AAC ATC ATG GTT CTG GTG AAC ACC TTT TGT GAC AGC TGC ATA CCC AAA ACC TTT TGG AAT CTG GGC   1587
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 Y   N   I   M   V   L   V   N   T   F   C   D   S   C   I   P   K   T   F   W   N   L   G     529

TAC TGG CTG TGC TAC ATC AAC AGC ACC GTG AAC CCC GTG TGC TAT GCT CTG TGC AAC AAA ACA TTC AGA   1656
 Y   W   L   C   Y   I   N   S   T   V   N   P   V   C   Y   A   L   C   N   K   T   F   R     552

ACC ACT TTC AAG ATG CTG CTG CTG TGC CAG TGT GAC AAA AAA AAG AGG CGC AAG CAG CAG TAC CAG CAG   1725
 T   T   F   K   M   L   L   L   C   Q   C   D   K   K   K   R   R   K   Q   Q   Y   Q   Q     575

AGA CAG TCG GTC ATT TTT CAC AAG CGC GCA CCC GAG CAG GCC TTG TAG
 R   Q   S   V   I   F   H   K   R   A   P   E   Q   A   L  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS116M	ChemiScreen™ M_3 Acetylcholine (Muscarinic) Family 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. Wess J (2004) Muscarinic acetylcholine knockout mice: novel phenotypes and clinical implications. *Annu. Rev. Pharmacol. Toxicol.* 44: 423-450.

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