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PRODUCT DATASHEET

Ready-to-Assay™ OT Oxytocin Receptor Frozen Cells

CATALOG NUMBER: HTS090RTA

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.

Oxytocin is a cyclic 9 amino acid peptide that differs from vasopressin in 2 amino acids. Despite the close similarities in sequence, oxytocin and vasopressin have different biological activities and bind to distinct G protein-coupled receptors. The oxytocin receptor, OT, couples primarily to Gq/11 to mobilize intracellular calcium. In female reproduction, oxytocin promotes uterine contraction and lactation; oxytocin is the most commonly used drug for induction of labor, whereas an oxytocin antagonist, atosiban, is under investigation to suppress preterm labor. Oxytocin/OT interaction in the CNS also plays an important role in stress, male and female sexual response, and sociality (Gimpl and Fahrenholz, 2001). Cloned human OT-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant OT 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 OT.

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

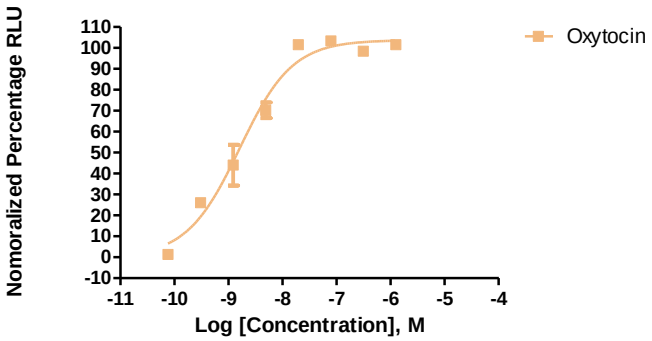

Figure 1. Representative data for activation of OT receptor. Calcium flux in OT–expressing Chem-1 cell line induced by Oxytocin. OT –expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed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

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

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Oxytocin	Calcium Flux	1.6	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
Oxytocin ligand	Sigma: O6379
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

OXTR cDNA (Accession Number: NM_000916; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG GAG GGC GCG CTC GCA GCC AAC TGG AGC GCC GAG
                                                 M   E   G   A   L   A   A   N   W   S   A   E

GCA GCC AAC GCC AGC GCC GCG CCG CCG GGG GCC GAG GGC AAC CGC ACC GCC GGA CCC CCG CGG CGC AAC
 A   A   N   A   S   A   A   P   P   G   A   E   G   N   R   T   A   G   P   P   R   R   N


GAG GCC CTG GCG CGC GTG GAG GTG GCG GTG CTG TGT CTC ATC CTG CTC CTG GCG CTG AGC GGG AAT GCG
 E   A   L   A   R   V   E   V   A   V   L   C   L   I   L   L   L   A   L   S   G   N   A

TGT GTG CTG CTG GCG CTG CGC ACC ACA CGC CAG AAG CAC TCG CGC CTC TTC TTC TTC ATG AAG CAC CTA
 C   V   L   L   A   L   R   T   T   R   Q   K   H   S   R   L   F   F   F   M   K   H   L

AGC ATC GCC GAC CTG GTG GTG GCA GTG TTT CAG GTG CTG CCG CAG TTG CTG TGG GAC ATC ACC TTC CGC
 S   I   A   D   L   V   V   A   V   F   Q   V   L   P   Q   L   L   W   D   I   T   F   R

TTC TAC GGG CCC GAC CTG CTG TGC CGC CTG GTC AAG TAC TTG CAG GTG GTG GGC ATG TTC GCC TCC ACC
 F   Y   G   P   D   L   L   C   R   L   V   K   Y   L   Q   V   V   G   M   F   A   S   T

TAC CTG CTG CTG CTC ATG TCC CTG GAC CGC TGC CTG GCC ATC TGC CAG CCG CTG CGC TCG CTG CGC CGC
 Y   L   L   L   L   M   S   L   D   R   C   L   A   I   C   Q   P   L   R   S   L   R   R

CGC ACC GAC CGC CTG GCA GTG CTC GCC ACG TGG CTC GGC TGC CTG GTG GCC AGC GCG CCG CAG GTG CAC
 R   T   D   R   L   A   V   L   A   T   W   L   G   C   L   V   A   S   A   P   Q   V   H

ATC TTC TCT CTG CGC GAG GTG GCT GAC GGC GTC TTC GAC TGC TGG GCC GTC TTC ATC CAG CCC TGG GGA
 I   F   S   L   R   E   V   A   D   G   V   F   D   C   W   A   V   F   I   Q   P   W   G

CCC AAG GCC TAC ATC ACA TGG ATC ACG CTA GCT GTC TAC ATC GTG CCG GTC ATC GTG CTC GCT GCC TGC
 P   K   A   Y   I   T   W   I   T   L   A   V   Y   I   V   P   V   I   V   L   A   A   C

TAC GGC CTT ATC AGC TTC AAG ATC TGG CAG AAT TTG CGG CTC AAG ACC GCT GCA GCG GCG GCG GCC GAG
 Y   G   L   I   S   F   K   I   W   Q   N   L   R   L   K   T   A   A   A   A   A   A   E

GCG CCA GAG GGC GCG GCG GCT GGC GAT GGG GGG CGC GTG GCC CTG GCG CGT GTC AGC AGC GTC AAG CTC
 A   P   E   G   A   A   A   G   D   G   G   R   V   A   L   A   R   V   S   S   V   K   L

ATC TCC AAG GCC AAG ATC CGC ACG GTC AAG ATG ACT TTC ATC ATC GTG CTG GCC TTC ATC GTG TGC TGG
 I   S   K   A   K   I   R   T   V   K   M   T   F   I   I   V   L   A   F   I   V   C   W

ACG CCT TTC TTC TTC GTG CAG ATG TGG AGC GTC TGG GAT GCC AAC GCG CCC AAG GAA GCC TCG GCC TTC
 T   P   F   F   F   V   Q   M   W   S   V   W   D   A   N   A   P   K   E   A   S   A   F

ATC ATC GTC ATG CTC CTG GCC AGC CTC AAC AGC TGC TGC AAC CCC TGG ATC TAC ATG CTG TTC ACG GGC
 I   I   V   M   L   L   A   S   L   N   S   C   C   N   P   W   I   Y   M   L   F   T   G

CAC CTC TTC CAC GAA CTC GTG CAG CGC TTC CTG TGC TGC TCC GCC AGC TAC CTG AAG GGC AGA CGC CTG
 H   L   F   H   E   L   V   Q   R   F   L   C   C   S   A   S   Y   L   K   G   R   R   L

GGA GAG ACG AGT GCC AGC AAA AAG AGC AAC TCG TCC TCC TTT GTC CTG AGC CAT CGC AGC TCC AGC CAG
 G   E   T   S   A   S   K   K   S   N   S   S   S   F   V   L   S   H   R   S   S   S   Q

AGG AGC TGC TCC CAG CCA TCC ACG GCG TGA
 R   S   C   S   Q   P   S   T   A  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS090M	ChemiScreen™ OT Oxytocin receptor membrane prep

REFERENCES

1. Gimpl G and Fahrenholz F (2001) The oxytocin receptor system:structure, function and regulation. *Physiol. Rev.* 81: 629-683.

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