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PRODUCT DATASHEET

ChemiScreen™ OT Oxytocin Receptor Stable Cell Line

CATALOG NUMBER: HTS090C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

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 $G_{q/11}$ 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 receptor-expressing ChemiScreen cells were constructed by stable transfection of Chem-1 cells with receptor and a promiscuous G protein to couple the receptor to the calcium signaling pathway. These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the OT receptor.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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 Fluorescence Assays

APPLICATION DATA

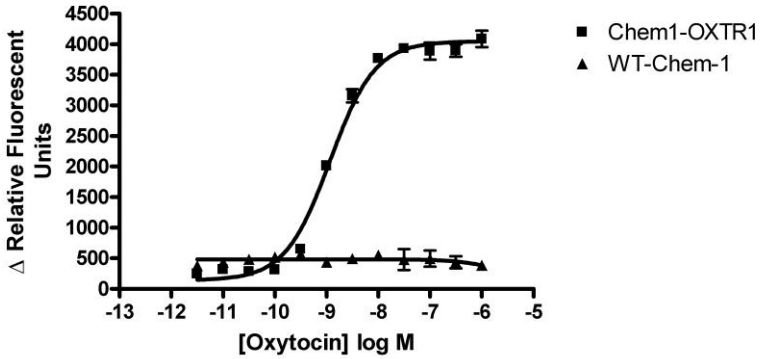

Figure 1. Representative data for activation of OT receptor stably expressed in Chem-1 cells induced by oxytocin using a fluorescent calcium flux assay. OT–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 3,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

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

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Oxytocin	Calcium Flux - Fluorescence	1.2	Eurofins Internal Data

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min*. Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.45	72

ASSAY SETUP

Fluorescence

Table 4. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Oxytocin ligand	Sigma: O6379
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve $5x10^5$cells/ml *(i.e, if collected 5e6 TC, $^{5e6/}_{5e5/ml}$ =10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human OT cDNA (Accession Number: NM_000916; see CODING SEQUENCE below)

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-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS090M	ChemiScreen™ OT Oxytocin family 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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