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PRODUCT DATASHEET

ChemiScreen™ D_1 Dopamine Receptor Stable Cell Line

CATALOG NUMBER: HTS102C

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-6 host, which supports high levels of functional receptor expression on the cell surface. Chem-6 cells contain high levels of promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway

Dopamine exerts its action by binding to five distinct dopamine receptors, all of which belong to G protein-coupled receptor family (Missale et al. 1998). The D_1 subtype is the most abundant dopamine receptor in the central nervous system. Activation of D_1 receptor stimulates adenylyl cyclase, activates cyclic AMP-dependent protein kinases. It regulates neuronal growth and development, mediate some behavioral responses and modulate dopamine receptor D_2-mediated events. The cloned human D_1 receptor-expressing ChemiScreen cells were constructed by stable transfection of Chem-6 cells with D_1. These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the D_1 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 Assay, cAMP accumulation

APPLICATION DATA

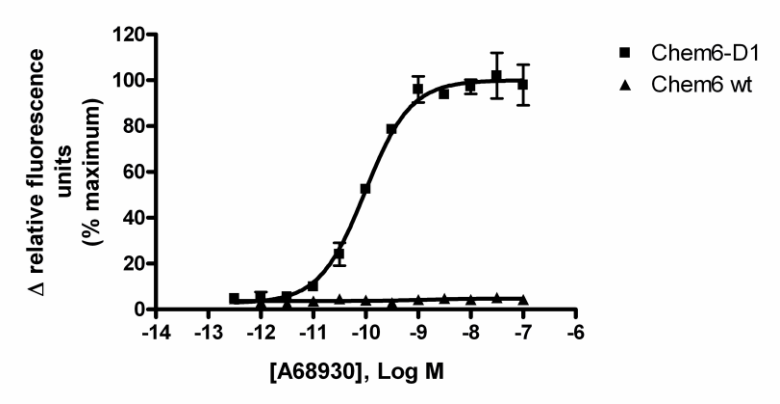

Figure 1. Representative data for activation of D_1 receptor stably expressed in Chem-6 cells induced by A68930 using a fluorescent calcium flux assay. D_1–expressing Chem-6 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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 12,000 RLU. Similarly parental cells (catalog #: DASFDSAFD) were tested to determine the specificity of the resulting signal.

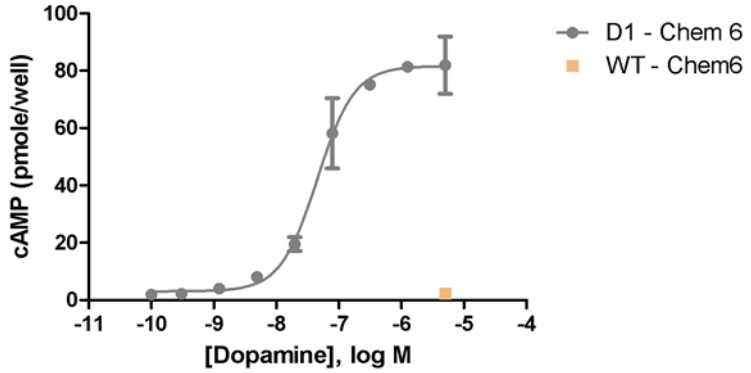

Figure 2. Representative data for activation of D_1 receptor stably expressed in CHEM-6 cells induced by Dopamine using a cAMP accumulation assay. D_1–expressing CHEM-1 cells were seeded at 100,000 cells per well into a 96-well plate, and the following day the cells were treated with Dopamine for 15 minutes in the presence of 2.0 mM IBMX and 0.5% DMSO to determine receptor-mediated cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Maximal cAMP response obtained in this experiment was 90 pmol/well. Similarly parental cells (catalog #: HTSCHEM-6) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of D_1-expressing Chem-6 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
A68930	Calcium Flux - Fluorescence	0.1	Eurofins Internal Data
Dopamine	cAMP accumulation	45	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	F-12 Kaighn's	-	Hyclone: SH3052601
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Gibco:10131-027
	Zeocin	200 µg/ml	Gibco: R25001
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^{TM}, AM	AAT Bioquest: 21080
Dopamine ligand	Sigma: H8502-5 g
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

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.

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HOST CELL

Chem-6, an adherent cell line expressing the promiscuous G-protein, Gα16.

EXOGENOUS GENE EXPRESSION

DRD1 cDNA (Accession Number: NM_000794; see CODING SEQUENCE below)

CODING SEQUENCE

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                                                  ATG AGG ACT CTG AAC ACC TCT GCC ATG GAC GGG
                                                   M   R   T   L   N   T   S   A   M   D   G
ACT GGG CTG GTG GTG GAG AGG GAC TTC TCT GTT CGT ATC CTC ACT GCC TGT TTC CTG TCG CTG CTC ATC
 T   G   L   V   V   E   R   D   F   S   V   R   I   L   T   A   C   F   L   S   L   L   I
CTG TCC ACG CTC CTG GGG AAC ACG CTG GTC TGT GCT GCC GTT ATC AGG TTC CGA CAC CTG CGG TCC AAG
 L   S   T   L   L   G   N   T   L   V   C   A   A   V   I   R   F   R   H   L   R   S   K
GTG ACC AAC TTC TTT GTC ATC TCC TTG GCT GTG TCA GAT CTC TTG GTG GCC GTC CTG GTC ATG CCC TGG
 V   T   N   F   F   V   I   S   L   A   V   S   D   L   L   V   A   V   L   V   M   P   W
AAG GCA GTG GCT GAG ATT GCT GGC TTC TGG CCC TTT GGG TCC TTC TGT AAC ATC TGG GTG GCC TTT GAC
 K   A   V   A   E   I   A   G   F   W   P   F   G   S   F   C   N   I   W   V   A   F   D
ATC ATG TGC TCC ACT GCA TCC ATC CTC AAC CTC TGT GTG ATC AGC GTG GAC AGG TAT TGG GCT ATC TCC
 I   M   C   S   T   A   S   I   L   N   L   C   V   I   S   V   D   R   Y   W   A   I   S
AGC CCT TTC CGG TAT GAG AGA AAG ATG ACC CCC AAG GCA GCC TTC ATC CTG ATC AGT GTG GCA TGG ACC
 S   P   F   R   Y   E   R   K   M   T   P   K   A   A   F   I   L   I   S   V   A   W   T
TTG TCT GTA CTC ATC TCC TTC ATC CCA GTG CAG CTC AGC TGG CAC AAG GCA AAA CCC ACA AGC CCC TCT
 L   S   V   L   I   S   F   I   P   V   Q   L   S   W   H   K   A   K   P   T   S   P   S
GAT GGA AAT GCC ACT TCC CTG GCT GAG ACC ATA GAC AAC TGT GAC TCC AGC CTC AGC AGG ACA TAT GCC
 D   G   N   A   T   S   L   A   E   T   I   D   N   C   D   S   S   L   S   R   T   Y   A
ATC TCA TCC TCT GTA ATA AGC TTT TAC ATC CCT GTG GCC ATC ATG ATT GTC ACC TAC ACC AGG ATC TAC
 I   S   S   S   V   I   S   F   Y   I   P   V   A   I   M   I   V   T   Y   T   R   I   Y
AGG ATT GCT CAG AAA CAA ATA CGG CGC ATT GCG GCC TTG GAG AGG GCA GCA GTC CAC GCC AAG AAT TGC
 R   I   A   Q   K   Q   I   R   R   I   A   A   L   E   R   A   A   V   H   A   K   N   C
CAG ACC ACC ACA GGT AAT GGA AAG CCT GTC GAA TGT TCT CAA CCG GAA AGT TCT TTT AAG ATG TCC TTC
 Q   T   T   T   G   N   G   K   P   V   E   C   S   Q   P   E   S   S   F   K   M   S   F
AAA AGA GAA ACT AAA GTC CTG AAG ACT CTG TCG GTG ATC ATG GGT GTG TTT GTG TGC TGT TGG CTA CCT
 K   R   E   T   K   V   L   K   T   L   S   V   I   M   G   V   F   V   C   C   W   L   P
TTC TTC ATC TTG AAC TGC ATT TTG CCC TTC TGT GGG TCT GGG GAG ACG CAG CCC TTC TGC ATT GAT TCC
 F   F   I   L   N   C   I   L   P   F   C   G   S   G   E   T   Q   P   F   C   I   D   S
AAC ACC TTT GAC GTG TTT GTG TGG TTT GGG TGG GCT AAT TCA TCC TTG AAC CCC ATC ATT TAT GCC TTT
 N   T   F   D   V   F   V   W   F   G   W   A   N   S   S   L   N   P   I   I   Y   A   F
AAT GCT GAT TTT CGG AAG GCA TTT TCA ACC CTC TTA GGA TGC TAC AGA CTT TGC CCT GCG ACG AAT AAT
 N   A   D   F   R   K   A   F   S   T   L   L   G   C   Y   R   L   C   P   A   T   N   N
GCC ATA GAG ACG GTG AGT ATC AAT AAC AAT GGG GCC GCG ATG TTT TCC AGC CAT CAT GAG CCA CGA GGC
 A   I   E   T   V   S   I   N   N   N   G   A   A   M   F   S   S   H   H   E   P   R   G
TCC ATC TCC AAG GAG TGC AAT CTG GTT TAC CTG ATC CCA CAT GCT GTG GGC TCC TCT GAG GAC CTG AAA
 S   I   S   K   E   C   N   L   V   Y   L   I   P   H   A   V   G   S   S   E   D   L   K
AAG GAG GAG GCA GCT GGC ATC GCC AGA CCC TTG GAG AAG CTG TCC CCA GCC CTA TCG GTC ATA TTG GAC
 K   E   E   A   A   G   I   A   R   P   L   E   K   L   S   P   A   L   S   V   I   L   D
TAT GAC ACT GAC GTC TCT CTG GAG AAG ATC CAA CCC ATC ACA CAA AAC GGT CAG CAC CCA ACC TGA
 Y   D   T   D   V   S   L   E   K   I   Q   P   I   T   Q   N   G   Q   H   P   T  Stp
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RELATED PRODUCTS

Product Number	Description
HTS102M	ChemiScreen™ D_1 Dopamine family receptor membrane prep

REFERENCES

1. Missale C *et al.* (1998) Dopamine receptors: from structure to function. *Physiol. Rev.* 78: 189-225.

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