eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ D_1 Dopamine Receptor Frozen Cells

CATALOG NUMBER: HTS102RTA

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.

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. Cloned human D_1-expressing cell line is made in the Chem-6 host, which supports high levels of recombinant D_1 expression and contains optimized levels of a recombinant promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at D_1.

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

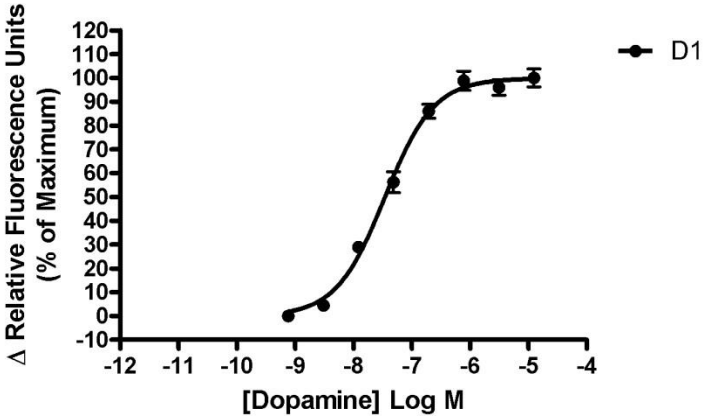

Figure 1. Representative data for activation of D_1 receptor. Calcium flux in D_1–expressing Chem-6 cell line induced by Dopamine. D_1–expressing Chem-6 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 4,200 RLU (Relative Light Units).

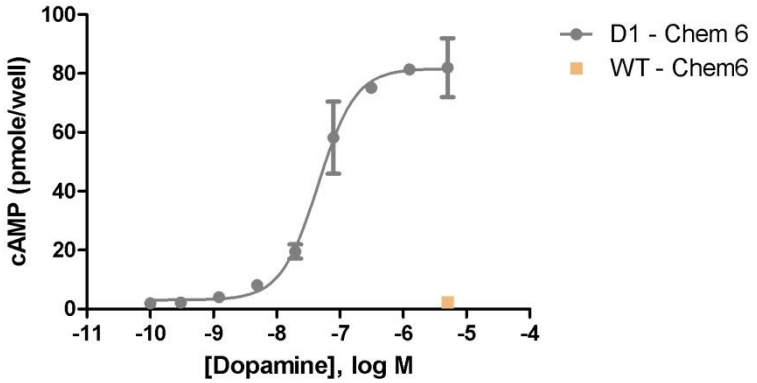

Figure 2. Representative data for activation of D1 receptor stably expressed in CHEM-6 cells induced by Dopamine using a cAMP accumulation assay. D1–expressing CHEM-6 cells were seeded at 100,000 cells per well into a 96-well plate, and the following day the cells were treated with Dopamine for 15 minutes 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} value of D_1-expressing Chem-6 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Dopamine	Calcium Flux	35	Eurofins Internal Data
Dopamine	cAMP Accumulation	45	Millipore 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
Dopamine ligand	Tocris: 1534
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-6, CHO-K1 cells expressing an exogenous proprietary promiscuous Gα protein.

EXONGENOUS GENE EXPRESSION

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

CODING SEQUENCE

```
                                                                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 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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