eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ D_5 Dopamine Receptor Frozen Cells

CATALOG NUMBER: HTS129RTA

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 is a catecholamine neurotransmitter that functions in the CNS to control locomotor, cognitive, emotional and neurendocrine processes, and in the periphery to modulate cardiovascular, renal and gastrointestinal processes. The biological activities of dopamine are mediated by a family of five GPCRs. The D_1 and D_5 subtypes couple to G_s to increase intracellular cAMP, whereas the D_2, D_3 and D_4 subtypes couple to G_i to reduce cAMP (Missale *et al*., 1998). The hypertensive phenotype of mice with a targeted deletion of D_5 indicates that D_5 regulates central control of sympathetic vascular tone (Hollon *et al*., 2002). In addition, D_5 modulates locomotion and corticostriatal long-term depression (Centonze *et al*., 2003). Cloned human D_5-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant D_5 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 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_5.

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 Fluorescent Assays, cAMP Accumulation Assays

APPLICATION DATA

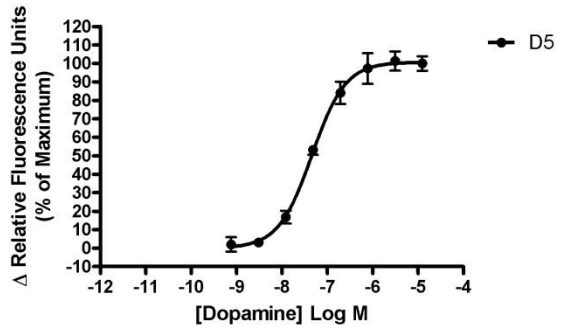

Figure 1. Representative data for activation of D_5 receptor. Calcium flux in D_5–expressing Chem-1 cell line induced by Dopamine. D_5–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 3,000 RLU (Relative Light Units).

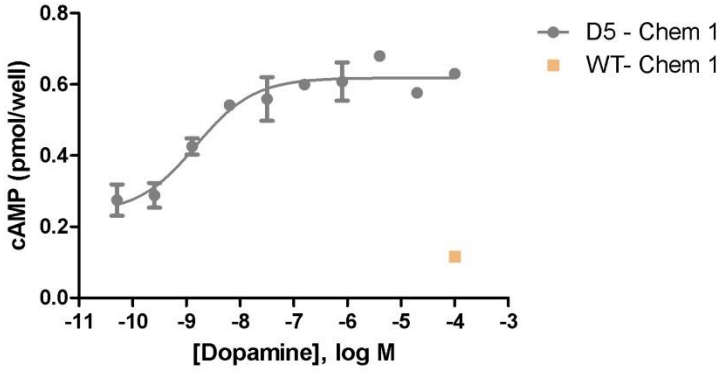

Figure 2. Representative data for activation of D_5 receptor stably expressed in CHEM-1 cells induced by Dopamine using a cAMP accumulation assay. D_5–expressing CHEM-1 cells were seeded at 100,000 cells per well into a 96-well plate, and the following day the cells were treated with Dopamine for 15 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 1pmol/well. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

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

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Dopamine	Calcium Flux	50	Eurofins Internal Data
Dopamine	cAMP Accumulation	1.5	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
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-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

EXONGENOUS GENE EXPRESSION

DRD5 cDNA (Accession Number: NM_000798; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                ATG CTG CCG CCA GGC AGC AAC GGC ACC GCG TAC CCG GGG CAG TTC GCT
                                  M   L   P   P   G   S   N   G   T   A   Y   P   G   Q   F   A
CTA TAC CAG CAG CTG GCG CAG GGG AAC GCC GTG GGG GGC TCG GCG GGG GCA CCG CCA CTG GGG CCC TCA
 L   Y   Q   Q   L   A   Q   G   N   A   V   G   G   S   A   G   A   P   P   L   G   P   S

CAG GTG GTC ACC GCC TGC CTG CTG ACC CTA CTC ATC ATC TGG ACC CTG CTG GGC AAC GTG CTG GTG TGC
 Q   V   V   T   A   C   L   L   T   L   L   I   I   W   T   L   L   G   N   V   L   V   C

GCA GCC ATC GTG CGG AGC CGC CAC CTG CGC GCC AAC ATG ACC AAC GTC TTC ATC GTG TCT CTG GCC GTG
 A   A   I   V   R   S   R   H   L   R   A   N   M   T   N   V   F   I   V   S   L   A   V

TCA GAC CTT TTC GTG GCG CTG CTG GTC ATG CCC TGG AAG GCA GTC GCC GAG GTG GCC GGT TAC TGG CCC
 S   D   L   F   V   A   L   L   V   M   P   W   K   A   V   A   E   V   A   G   Y   W   P

TTT GGA GCG TTC TGC GAC GTC TGG GTG GCC TTC GAC ATC ATG TGC TCC ACT GCC TCC ATC CTG AAC CTG
 F   G   A   F   C   D   V   W   V   A   F   D   I   M   C   S   T   A   S   I   L   N   L

TGC GTC ATC AGC GTG GAC CGC TAC TGG GCC ATC TCC AGG CCC TTC CGC TAC AAG CGC AAG ATG ACT CAG
 C   V   I   S   V   D   R   Y   W   A   I   S   R   P   F   R   Y   K   R   K   M   T   Q

CGC ATG GCC TTG GTC ATG GTC GGC CTG GCA TGG ACC TTG TCC ATC CTC ATC TCC TTC ATT CCG GTC CAG
 R   M   A   L   V   M   V   G   L   A   W   T   L   S   I   L   I   S   F   I   P   V   Q

CTC AAC TGG CAC AGG GAC CAG GCG GCC TCT TGG GGC GGG CTG GAC CTG CCA AAC AAC CTG GCC AAC TGG
 L   N   W   H   R   D   Q   A   A   S   W   G   G   L   D   L   P   N   N   L   A   N   W

ACG CCC TGG GAG GAG GAC TTT TGG GAG CCC GAC GTG AAT GCA GAG AAC TGT GAC TCC AGC CTG AAT CGA
 T   P   W   E   E   D   F   W   E   P   D   V   N   A   E   N   C   D   S   S   L   N   R

ACC TAC GCC ATC TCT TCC TCG CTC ATC AGC TTC TAC ATC CCC GTT GCC ATC ATG ATC GTG ACC TAC ACG
 T   Y   A   I   S   S   S   L   I   S   F   Y   I   P   V   A   I   M   I   V   T   Y   T

CGC ATC TAC CGC ATC GCC CAG GTG CAG ATC CGC AGG ATT TCC TCC CTG GAG AGG GCC GCA GAG CAC GCG
 R   I   Y   R   I   A   Q   V   Q   I   R   R   I   S   S   L   E   R   A   A   E   H   A

CAG AGC TGC CGG AGC AGC GCA GCC TGC GCG CCC GAC ACC AGC CTG CGC GCT TCC ATC AAG AAG GAG ACC
 Q   S   C   R   S   S   A   A   C   A   P   D   T   S   L   R   A   S   I   K   K   E   T

AAG GTT CTC AAG ACC CTG TCG GTG ATC ATG GGG GTC TTC GTG TGT TGC TGG CTG CCC TTC TTC ATC CTT
 K   V   L   K   T   L   S   V   I   M   G   V   F   V   C   C   W   L   P   F   F   I   L
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 N   C   M   V   P   F   C   S   G   H   P   E   G   P   P   A   G   F   P   C   V   S   E

ACC ACC TTC GAC GTC TTC GTC TGG TTC GGC TGG GCT AAC TCC TCA CTC AAC CCC GTC ATC TAT GCC TTC
 T   T   F   D   V   F   V   W   F   G   W   A   N   S   S   L   N   P   V   I   Y   A   F

AAC GCC GAC TTT CAG AAG GTG TTT GCC CAG CTG CTG GGG TGC AGC CAC TTC TGC TCC CGC ACG CCG GTG
 N   A   D   F   Q   K   V   F   A   Q   L   L   G   C   S   H   F   C   S   R   T   P   V

GAG ACG GTG AAC ATC AGC AAT GAG CTC ATC TCC TAC AAC CAA GAC ATC GTC TTC CAC AAG GAA ATC GCA
 E   T   V   N   I   S   N   E   L   I   S   Y   N   Q   D   I   V   F   H   K   E   I   A

GCT GCC TAC ATC CAC ATG ATG CCC AAC GCC GTT ACC CCC GGC AAC CGG GAG GTG GAC AAC GAC GAG GAG
 A   A   Y   I   H   M   M   P   N   A   V   T   P   G   N   R   E   V   D   N   D   E   E

GAG GGT CCT TTC GAT CGC ATG TTC CAG ATC TAT CAG ACG TCC CCA GAT GGT GAC CCT GTT GCT GAG TCT
 E   G   P   F   D   R   M   F   Q   I   Y   Q   T   S   P   D   G   D   P   V   A   E   S

GTC TGG GAG CTG GAC TGC GAG GGG GAG ATT TCT TTA GAC AAA ATA ACA CCT TTC ACC CCG AAT GGA TTC
 V   W   E   L   D   C   E   G   E   I   S   L   D   K   I   T   P   F   T   P   N   G   F

CAT TGA
 H  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS129M	ChemiScreen™ D_5 Dopamine receptor membrane prep

REFERENCES

1. Centonze D *et al.* (2003) Distinct roles of D_1 and D_5 dopamine receptors in motor activity and striatal synaptic plasticity. *J. Neurosci.* 23: 8506-8512.
2. Hollon TR *et al*. (2002) Mice lacking D_5 dopamine receptors have increased sympathetic tone and are hypertensive. *J. Neurosci.* 22: 10801-10810.
3. Missale C *et al.* (1998) Dopamine receptors: from structure to function. *Physiol. Rev.* 78: 189-225.

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