eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ D_{2L} Dopamine Receptor Frozen Cells

CATALOG NUMBER: HTS039RTA

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 D_2 dopamine receptors have been of particular clinical interest due to their regulation of prolactin secretion and their affinity for antipsychotic drugs. The D_2 receptor exists as two alternatively spliced isoforms differing in the insertion of a stretch of 29 amino acids in the third intracellular loop (D_{2S} and D_{2L}) (Giros *et al.*, 1989; Grandy *et al.*, 1989). Cloned human D_{2L}-expressing cell line is made in the Chem-7 host, which supports high levels of recombinant D_{2L} 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_{2L}.

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

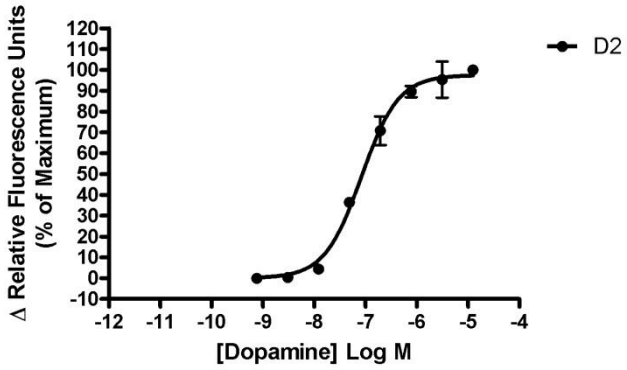

Figure 1. Representative data for activation of D_{2L} receptor. Calcium flux in D_{2L}–expressing Chem-7 cell line induced by Dopamine. D_{2L}–expressing Chem-7 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 8,000 RLU (Relative Light Units).

Table 1. EC_{50} value of D_{2L}-expressing Chem-7 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Dopamine	Calcium Flux	80	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	Sigma: H8502
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-7, a CHO-K1 cell line expressing a proprietary exogenous Gα protein

EXONGENOUS GENE EXPRESSION

DRD2 cDNA (Accession Number: NM_000795; see CODING SEQUENCE below).

CODING SEQUENCE

```
   1 - ATG GAT CCA CTG AAT CTG TCC TGG TAT GAT GAT GAT CTG GAG AGG CAG AAC TGG AGC CGG  - 60
   1 -  M   D   P   L   N   L   S   W   Y   D   D   D   L   E   R   Q   N   W   S   R  - 20

  61 - CCC TTC AAC GGG TCA GAC GGG AAG GCG GAC AGA CCC CAC TAC AAC TAC TAT GCC ACA CTG  - 120
  21 -  P   F   N   G   S   D   G   K   A   D   R   P   H   Y   N   Y   Y   A   T   L  - 40

 121 - CTC ACC CTG CTC ATC GCT GTC ATC GTC TTC GGC AAC GTG CTG GTG TGC ATG GCT GTG TCC  - 180
  41 -  L   T   L   L   I   A   V   I   V   F   G   N   V   L   V   C   M   A   V   S  - 60

 181 - CGC GAG AAG GCG CTG CAG ACC ACC ACC AAC TAC CTG ATC GTC AGC CTC GCA GTG GCC GAC  - 240
  61 -  R   E   K   A   L   Q   T   T   T   N   Y   L   I   V   S   L   A   V   A   D  - 80

 241 - CTC CTC GTC GCC ACA CTG GTC ATG CCC TGG GTT GTC TAC CTG GAG GTG GTA GGT GAG TGG  - 300
  81 -  L   L   V   A   T   L   V   M   P   W   V   V   Y   L   E   V   V   G   E   W  - 100

 301 - AAA TTC AGC AGG ATT CAC TGT GAC ATC TTC GTC ACT CTG GAC GTC ATG ATG TGC ACG GCG  - 360
 101 -  K   F   S   R   I   H   C   D   I   F   V   T   L   D   V   M   M   C   T   A  - 120

 361 - AGC ATC CTG AAC TTG TGT GCC ATC AGC ATC GAC AGG TAC ACA GCT GTG GCC ATG CCC ATG  - 420
 121 -  S   I   L   N   L   C   A   I   S   I   D   R   Y   T   A   V   A   M   P   M  - 140

 421 - CTG TAC AAT ACG CGC TAC AGC TCC AAG CGC CGG GTC ACC GTC ATG ATC TCC ATC GTC TGG  - 480
 141 -  L   Y   N   T   R   Y   S   S   K   R   R   V   T   V   M   I   S   I   V   W  - 160

 481 - GTC CTG TCC TTC ACC ATC TCC TGC CCA CTC CTC TTC GGA CTC AAT AAC GCA GAC CAG AAC  - 540
 161 -  V   L   S   F   T   I   S   C   P   L   L   F   G   L   N   N   A   D   Q   N  - 180

 541 - GAG TGC ATC ATT GCC AAC CCG GCC TTC GTG GTC TAC TCC TCC ATC GTC TCC TTC TAC GTG  - 600
 181 -  E   C   I   I   A   N   P   A   F   V   V   Y   S   S   I   V   S   F   Y   V  - 200

 601 - CCC TTC ATT GTC ACC CTG CTG GTC TAC ATC AAG ATC TAC ATT GTC CTC CGC AGA CGC CGC  - 660
 201 -  P   F   I   V   T   L   L   V   Y   I   K   I   Y   I   V   L   R   R   R   R  - 220

 661 - AAG CGA GTC AAC ACC AAA CGC AGC AGC CGA GCT TTC AGG GCC CAC CTG AGG GCT CCA CTA  - 720
 221 -  K   R   V   N   T   K   R   S   S   R   A   F   R   A   H   L   R   A   P   L  - 240

 721 - AAG GGC AAC TGT ACT CAC CCC GAG GAC ATG AAA CTC TGC ACC GTT ATC ATG AAG TCT AAT  - 780
 241 -  K   G   N   C   T   H   P   E   D   M   K   L   C   T   V   I   M   K   S   N  - 260

 781 - GGG AGT TTC CCA GTG AAC AGG CGG AGA GTG GAG GCT GCC CGG CGA GCC CAG GAG CTG GAG  - 840
 261 -  G   S   F   P   V   N   R   R   R   V   E   A   A   R   R   A   Q   E   L   E  - 280

 841 - ATG GAG ATG CTC TCC AGC ACC AGC CCA CCC GAG AGG ACC CGG TAC AGC CCC ATC CCA CCC  - 900
 281 -  M   E   M   L   S   S   T   S   P   P   E   R   T   R   Y   S   P   I   P   P  - 300

 901 - AGC CAC CAC CAG CTG ACT CTC CCC GAC CCG TCC CAC CAT GGT CTC CAC AGC ACT CCT GAC  - 960
 301 -  S   H   H   Q   L   T   L   P   D   P   S   H   H   G   L   H   S   T   P   D  - 320

 961 - AGC CCC GCC AAA CCA GAG AAG AAT GGG CAT GCC AAA GAC CAC CCC AAG ATT GCC AAG ATC  - 1020
 321 -  S   P   A   K   P   E   K   N   G   H   A   K   D   H   P   K   I   A   K   I  - 340

1021 - TTT GAG ATC CAG ACC ATG CCC AAT GGC AAA ACC CGG ACC TCC CTC AAG ACC ATG AGC CGT  - 1080
 341 -  F   E   I   Q   T   M   P   N   G   K   T   R   T   S   L   K   T   M   S   R  - 360

1081 - AGG AAG CTC TCC CAG CAG AAG GAG AAG AAA GCC ACT CAG ATG CTC GCC ATT GTT CTC GGC  - 1140
 361 -  R   K   L   S   Q   Q   K   E   K   K   A   T   Q   M   L   A   I   V   L   G  - 380

1141 - GTG TTC ATC ATC TGC TGG CTG CCC TTC TTC ATC ACA CAC ATC CTG AAC ATA CAC TGT GAC  - 1200
 381 -  V   F   I   I   C   W   L   P   F   F   I   T   H   I   L   N   I   H   C   D  - 400

1201 - TGC AAC ATC CCG CCT GTC CTG TAC AGC GCC TTC ACG TGG CTG GGC TAT GTC AAC AGC GCC  - 1260
 401 -  C   N   I   P   P   V   L   Y   S   A   F   T   W   L   G   Y   V   N   S   A  - 420

1261 - GTG AAC CCC ATC ATC TAC ACC ACC TTC AAC ATT GAG TTC CGC AAG GCC TTC CTG AAG ATC  - 1320
 421 -  V   N   P   I   I   Y   T   T   F   N   I   E   F   R   K   A   F   L   K   I  - 440

1321 - CTT CAC TGC TGA
 441 -  L   H   C  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTS039M	ChemiScreen™ D_{2L} Dopamine receptor membrane prep

REFERENCES

1. Grandy DK *et al.* (1989) Cloning of the cDNA and gene for a human D2 dopamine receptor. Proc Natl Acad Sci U S A.. 86:9762-6.
2. Giros B *et al.* (1989) Alternative splicing directs the expression of two D2 dopamine receptor isoforms. Nature. 342:923-6.
3. Missale C *et al.* (1998) Dopamine receptors: from structure to function. *Physiol. Rev.* 78: 189-225.

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