

## PRODUCT DATASHEET

### Ready-to-Assay™ D<sub>4</sub> Dopamine Receptor Frozen Cells

#### CATALOG NUMBER: HTS223RTA

**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<sub>2</sub>. Media Component at 4°C (-20°C 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.

The neurotransmitter dopamine is involved in a wide variety of neuroendocrine, emotional, cognitive and locomotor functions. These activities of dopamine are mediated by a group of 5 G protein coupled receptors, 2 of which (D<sub>1</sub> and D<sub>5</sub>) couple to G<sub>s</sub> and 3 of which (D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>) couple to G<sub>i</sub> (Missale *et al.*, 1998). The D<sub>4</sub> receptor is highly polymorphic, particularly in the third cytoplasmic loop, which contains from 2 to 7 repeats of 16 amino acids. Individuals with 7 repeats have increased risk of developing ADHD (Thapar *et al.*, 2005). Mice engineered to lack D<sub>4</sub> have reduced locomotor activity, increased sensitivity to drugs of abuse that elevate dopamine (cocaine, methamphetamine and ethanol), and reduced response to novelty (Rubinstein *et al.*, 1997; Dulawa *et al.*, 1999). Cloned human D<sub>4</sub>-expressing cell line is made in the Chem-5 host, which supports high levels of recombinant D<sub>4</sub> expression on the cell surface 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<sub>4</sub>.

#### 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.

## APPLICATIONS

Calcium Flux Assays

### APPLICATION DATA

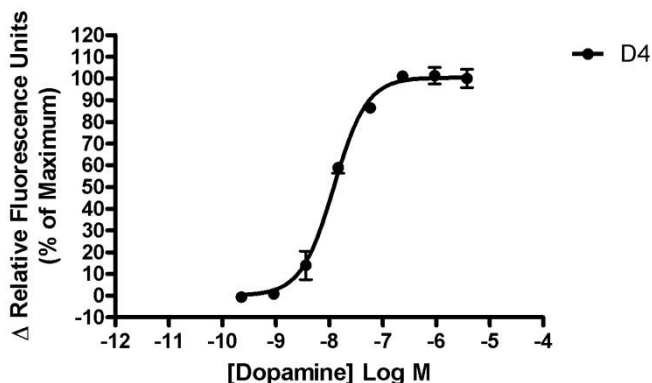


Figure 1. Representative data for activation of D<sub>4</sub> receptor. Calcium flux in D<sub>4</sub>-expressing Chem-5 cell line induced by Dopamine. D<sub>4</sub>-expressing Chem-5 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR<sup>TETRA</sup> with ICCD camera. Maximal fluorescence signal obtained in this experiment was 11,600 RLU (Relative Light Units).

Table 1. EC<sub>50</sub> values of D<sub>4</sub>-expressing Chem-5 cells

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Dopamine	Calcium Flux	10	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 37°C 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 37°C 5% CO<sub>2</sub> 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<sup>2+</sup> dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca<sup>2+</sup> dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca<sup>2+</sup> 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<sup>TETRA</sup>) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR<sup>TETRA</sup>) or emission filter for Ca<sup>2+</sup> 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
Probenecid	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<sup>TETRA</sup>® 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-5, an adherent rat hematopoietic cell line expressing endogenous G·15 protein as well as an exogenous proprietary promiscuous Gα protein.

## EXONGENOUS GENE EXPRESSION

DRD4.4 cDNA (Accession Number: NM\_000797; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

### CODING SEQUENCE

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      ATGGGGAACCGCAGCACCGCGGACGCGGACGGGTGCTGGCTGGGCGCGGGCCCGCGGGGGCATCTGCGGGGGCATCTGCGGGGCTG - 90
1 - M G N R S T A D A D G L L A G R G P A A G A S A G A S A G L - 30
      GCTGGGCAGGCGCGGGCGGCTGGTGGGGGGCTGCTCATCGGCGCGGTGCTCGCGGGAACTCGTCTGTCGTGAGCGTGGCC - 180
31 - A G Q G A A A L V G G V L L I G A V L A G N S L V C V S V A - 60
      ACCGAGCGCGCCCTGCAGACGCCCACTCCTTCATCGTGAGCCTGGGCGCGCGACCTCCTCCTCGTCTCCTGGTGTGCGCGTC - 270
61 - T E R A L Q T P T N S F I V S L A A A D L L L A L L V L P L - 90
      TTCGTCTACTCCGAGGTCCAGGGTGGCGGTGGCTGCTGAGCCCCCGCTGTCGACGCCCTCATGGCCATGGACGTGTCGTGTCACC - 360
91 - F V Y S E V Q G G A W L L S P R L C D A L M A M D V M L C T - 120
      GCCTCCATCTTCAACCTGTGCGCCATCAGCGTGGACAGTTCGTGGCCGTGGCCGTGCCGTGCGCTACAACGGCAGGGTGGGAGCGCG - 450
121 - A S I F N L C A I S V D R F V A V A V P L R Y N R Q G G S R - 150
      CGGCAGCTGTGCTCATCGGCGCCACGTGGCTGCTGTCGCGCGGTGGGCGCGCCGCTACTGTGCGGCCCAACGACGTGCGCGGCGCG - 540
151 - R Q L L L I G A T W L L S A A V A A P V L C G L N D V R G R - 180
      GACCCCGCGTGTGCCGCTGGAGGACCGGACTACGTGGTCTACTCGTCCGTGTGCTCCTTCTCCTACCCCTGCCCGCTCATGCTGCTG - 630
181 - D P A V C R L E D R D Y V V Y S S V C S F F L P C P L M L L - 210
      CTCTACTGGGCCACGTTCCGCGCCTGCAGCGTGGGAGGTGGCACGTCGCGCCAAGCTGCACGGCCGCGCGCCCGCCGACCCAGCGGC - 720
211 - L Y W A T F R G L Q R W E V A R R A K L H G R A P R R P S G - 240
      CCTGGCCCGCTTCCCCACGCCACCCGCGCCCGCTCCCCAGGACCCCTGCGGCCCCGACTGTGCGCCCCCGCGCCCGGCTTCCC - 810
241 - P G P P S P T P P A P R L P Q D P C G P D C A P P A P G L P - 270
      CGGGTCCCTGCGGCCCGACTGTGCGCCCGCGCGCCAGCCCTCCCCAGGACCCCTGTGGCCCCGACTGTGCGCCCCCGCGCCCGGC - 900
271 - R G P C G P D C A P A A P S L P Q D P C G P D C A P P A P G - 300
      CTCGCCCGGACCCCTGCGGCTCCAACCTGTGCTCCCCCGACGCGCTCAGAGCCGCGCGCTCCCACCCAGACTCCACCGCAGACCCGC - 990
301 - L P P D P C G S N C A P P D A V R A A A L P P Q T P P Q T R - 330
      AGGAGCGCGGTGCCAAGATCACCGCGCGGAGCGCAAGGCCATGAGGGTCCCTGCCGGTGGTGGTGGGGCCCTTCCTGCTGTGCTGGACG -1080
331 - R R R R A K I T G R E R K A M R V L P V V V G A F L L C W T - 360
      CCCTTCTCGTGGTGCACATCACGACGGCGCTGTGTCCTGCCTGCTCCGTGCCCCCGCGGCTGGTACGCGCGTACCTGGCTGGGTAC -1170
361 - P F F V V H I T Q A L C P A C S V P P R L V S A V T W L G Y - 390
      GTCAACAGCGCCCTCAACCCCGTACATCTACTGTCTTCAACGCCGAGTTCGCAACGCTTCCGCAAGGCCCTGCGTGCCTGCTGCTGA -1260
391 - V N S A L N P V I Y T V F N A E F R N V F R K A L R A C C Stp - 420

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## RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
<b>HTSCHEM-1RTA</b>	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
<b>HTS223M</b>	ChemiScreen™ D <sub>4</sub> Dopamine receptor membrane prep

Note: Chem-5 cells are derived from Chem-1 cells.

## REFERENCES

1. Chemel BR *et al.* (2006) WAY-100635 is a potent dopamine D<sub>4</sub> receptor agonist. *Psychopharmacology* 188: 244-251.
2. Dulawa SC *et al.* (1999) Dopamine D<sub>4</sub> receptor-knock-out mice exhibit reduced exploration of novel stimuli. *J. Neurosci.* 19: 9550-9556.
3. Millan MJ *et al.* (1998) S 18126 ([2-[4-(2,3-dihydrobenzo[1,4]dioxin-6-yl)piperazin-1-yl methyl]indan-2-yl]), a potent, selective and competitive antagonist at dopamine D<sub>4</sub> receptors: an in vitro and in vivo comparison with L 745,870 (3-(4-[4-chlorophenyl]piperazin-1-yl)methyl-1H-pyrrolo[2, 3b]pyridine) and raclopride. *J. Pharmacol Exp. Ther.* 287: 167-186.
4. Missale C *et al.* (1998) Dopamine receptors: From structure to function. *Physiol. Rev.* 78:

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