User Manual

Ready-to-Assay™ D4 Dopamine Receptor Frozen Cells

HTS223RTA

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Not for use in diagnostic procedures. Not for Human or Animal Consumption

Product Overview

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. The freezing conditions have been optimized 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_1 and D_5) couple to Gs and 3 of which (D_2, D_3, D_4) couple to G_i (Missale et al., 1998). The D_4 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_4 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_4-expressing cell line is made in the Chem-5 host, which supports high levels of recombinant D_4 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_4.

Materials Provided

- Pack contains 2 vials of mycoplasma-free cells, 1 mL per vial. Store in liquid N_2.
- 50 mL of Media Component. Store at 4 °C (-20°C for prolonged storage).

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.

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

Applications Data

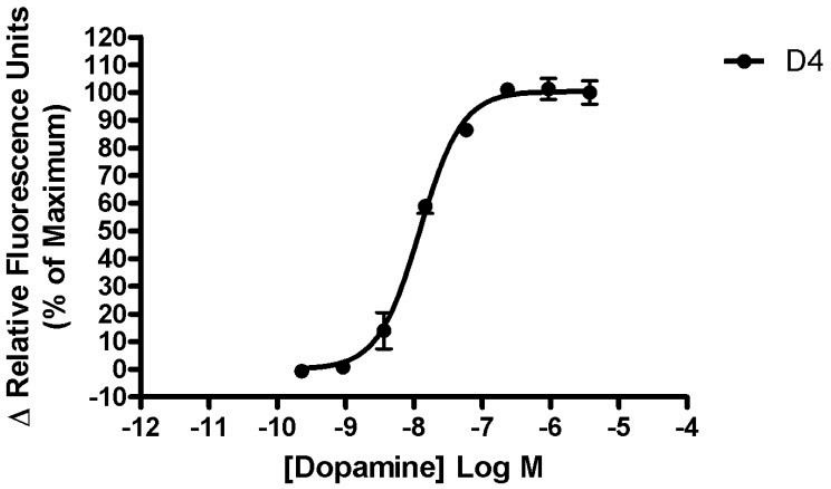

Figure 1. Representative data for activation of D4 receptor. Calcium flux in D_4 –expressing Chem-5 cell line induced by Dopamine. D_4 –expressing Chem-5 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR Tetra® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 11,600 RLU (Relative Light Units).

Table 1. EC_{50} values of D4 -expressing Chem-5 cells

Ligand	Assay	Potency (nM)	Reference
Dopamine	Calcium Flux	10	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_2 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 20 mM HEPES, 2.5 mM 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 20 mM HEPES, 2.5 mM 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

(FLIPRTETRA) 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

All items may be purchased at SigmaAldrich.com unless otherwise noted.

Description	Catalogue Number
HBSS	SH30268.02 (Hyclone)
HEPES 1M Stock	TMS-003-C
Probenicid	P8761
Quest Fluo-8™, AM	21080 (AAT Bioquest)
Dopamine ligand	H8502
Non-binding white plates (for ligand prep)	3605 (96-well)/3574 (384-well) (Corning)
Black (clear bottom) tissue-culture treated plates	3904 (96-well)/3712 (384-well) (Corning)

FLIPR Settings

Settings for FLIPR$^{TETRA®}$ with ICCD camera option.

Read Mode	Fluorescence
Ex/Em	Ex470_495/Em515_575
Camera Gain	2000
Gate Open	6%
Exposure Time	0.53
Read Interval	1 second
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 endogeneous G-15 protein as well as an exogenous proprietary promiscuous Gα protein.

Exogenous 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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      ATGGGGAACCGCAGCACCGCGGACGCGGACGGGCTGCTGGCTGGGCGCGGGCCGGCCGCGGGGGCATCTGCGGGGGCATCTGCGGGGCTG - 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

 91 - GCTGGGCAGGGCGCGGCGGCGCTGGTGGGGGGCGTGCTGCTCATCGGCGCGGTGCTCGCGGGGAACTCGCTCGTGTGCGTGAGCGTGGCC - 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

181 - ACCGAGCGCGCCCTGCAGACGCCCACCAACTCCTTCATCGTGAGCCTGGCGGCCGCCGACCTCCTCCTCGCTCTCCTGGTGCTGCCGCTC - 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

271 - TTCGTCTACTCCGAGGTCCAGGGTGGCGCGTGGCTGCTGAGCCCCCGCCTGTGCGACGCCCTCATGGCCATGGACGTCATGCTGTGCACC - 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

361 - GCCTCCATCTTCAACCTGTGCGCCATCAGCGTGGACAGGTTCGTGGCCGTGGCCGTGCCGCTGCGCTACAACCGGCAGGGTGGGAGCCGC - 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

451 - CGGCAGCTGCTGCTCATCGGCGCCACGTGGCTGCTGTCCGCGGCGGTGGCGGCGCCCGTACTGTGCGGCCTCAACGACGTGCGCGGCCGC - 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

541 - GACCCCGCCGTGTGCCGCCTGGAGGACCGCGACTACGTGGTCTACTCGTCCGTGTGCTCCTTCTTCCTACCCTGCCCGCTCATGCTGCTG - 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

631 - CTCTACTGGGCCACGTTCCGCGGCCTGCAGCGCTGGGAGGTGGCACGTCGCGCCAAGCTGCACGGCCGCGCGCCCCGCCGACCCAGCGGC - 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

721 - CCTGGCCCGCCTTCCCCCACGCCACCCGCGCCCCGCCTCCCCCAGGACCCCTGCGGCCCCGACTGTGCGCCCCCCGCGCCCGGCCTTCCC - 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

811 - CGGGGTCCCTGCGGCCCCGACTGTGCGCCCGCCGCGCCCAGCCTCCCCCAGGACCCCTGTGGCCCCGACTGTGCGCCCCCCGCGCCCGGC - 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

901 - CTCCCCCCGGACCCCTGCGGCTCCAACTGTGCTCCCCCCGACGCCGTCAGAGCCGCCGCGCTCCCACCCCAGACTCCACCGCAGACCCGC - 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

991 - AGGAGGCGGCGTGCCAAGATCACCGGCCGGGAGCGCAAGGCCATGAGGGTCCTGCCGGTGGTGGTCGGGGCCTTCCTGCTGTGCTGGACG -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

1081 - CCCTTCTTCGTGGTGCACATCACGCAGGCGCTGTGTCCTGCCTGCTCCGTGCCCCCGCGGCTGGTCAGCGCCGTCACCTGGCTGGGCTAC -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

1171 - GTCAACAGCGCCCTCAACCCCGTCATCTACACTGTCTTCAACGCCGAGTTCCGCAACGTCTTCCGCAAGGCCCTGCGTGCCTGCTGCTGA -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

- HTSCHEM-1RTA Ready-to-Assay™ Chem-1 host frozen cells (control cells).
  Note: Chem-5 cells are derived from Chem-1 cells.
- HTS223M ChemiScreen™ D4 Dopamine receptor membrane Preparation Recombinant Human D4 Dopamine Receptor.

References

1. Chemel BR et al. (2006) WAY-100635 is a potent dopamine D4 receptor agonist. Psychopharmacology 188: 244-251.
2. Dulawa SC et al. (1999) Dopamine D4 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 D4 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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