

PRODUCT DATASHEET

ChemiScreen™ D₅ Dopamine Receptor Stable Cell Line

CATALOG NUMBER: HTS129C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N₂.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

Dopamine is a catecholamine neurotransmitter that functions in the CNS to control locomotor, cognitive, emotional and neuroendocrine 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 D₁ and D₅ subtypes couple to G_s to increase intracellular cAMP, whereas the D₂, D₃ and D₄ subtypes couple to G_i to reduce cAMP (Missale et al., 1998). The hypertensive phenotype of mice with a targeted deletion of D₅ indicates that D₅ regulates central control of sympathetic vascular tone (Hollon et al., 2002). In addition, D₅ modulates locomotion and corticostriatal long-term depression (Centonze et al., 2003). The cloned human D₅-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant D₅ 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 antagonists of interactions between D₅ and its ligands.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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 Fluorescence Assay, cAMP accumulation

APPLICATION DATA

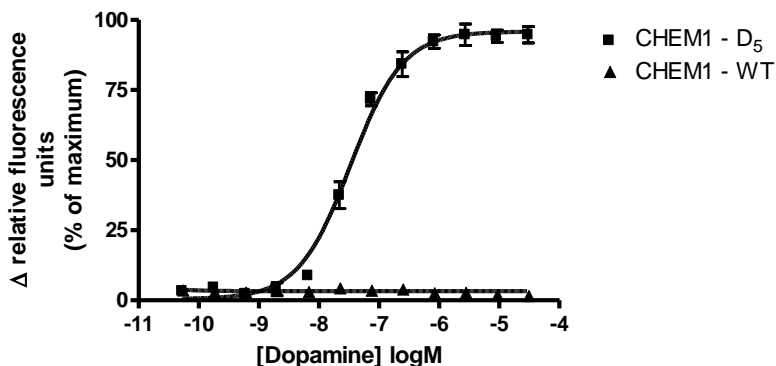


Figure 1. Representative data for activation of the D₅ receptor stably expressed in Chem-1 cells induced by Dopamine using a fluorescent calcium flux assay. D₅-expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

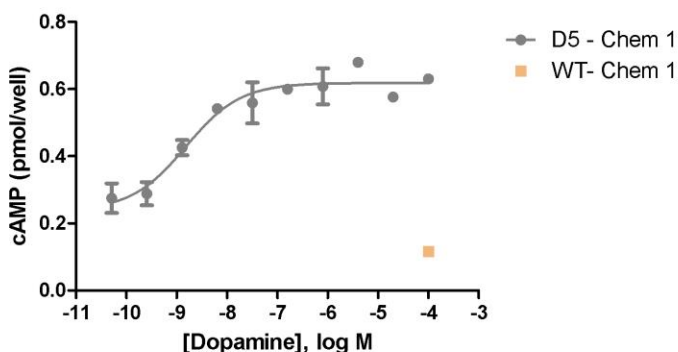


Figure 2. Representative data for activation of D₅ receptor stably expressed in CHEM-1 cells induced by Dopamine using a cAMP accumulation assay. D₅-expressing CHEM-1 cells were seeded at 100,000 cells per well into a 96-well plate, and the following day the cells were treated with Dopamine for 15 minutes 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. EC₅₀ values of D₅-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC ₅₀ (nM)	REFERENCE
Dopamine	Calcium Flux	33	Eurofins Internal Data
Dopamine	cAMP Accumulation	1.5	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC₅₀ and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	10mM	Millipore Sigma: H3537
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Gibco:10131-027
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37°C Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37°C water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37°C, 5% CO₂.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37° C incubator for additional 2 min.* Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm ²)	Volume (mL)	Total Cell Number (x10 ⁶)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.45	72

ASSAY SETUP

Fluorescence

 Table 4. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8 ^{IM} , AM	AAT Bioquest: 21080
Dopamine	Sigma: H8502
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712

cAMP

Table 6. Settings for Plate Reader

Option	Setting
Excitation	300 nm
Emission	665/620 nm

Table 7. cAMP Assay Materials (Not provided)

Description	Supplier and Product Number
HEPES 1M Stock	Millipore Sigma: H3537
IBMX Buffer	Sigma #15879
96-Well Flat Bottom Microtiter Plates	Costar #3917
Non-Binding 96 well Plates (for ligand prep)	Costar: #3789
Dopamine	Sigma: H8502
cAMP Hi Range Kit	CisBio # 62AM6PEC

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve 5×10^5 cells/ml (i.e, if collected 5×10^6 TC, $\frac{5 \times 10^6}{5 \times 10^5/ml} = 10$ mL volume)
4. Seed cell suspension into black, clear bottom plate (100 μ L/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37°C 5% CO₂ incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate by quickly inverting plate on an absorbent pad and blotting to remove all Media Component.
8. Add Loading buffer to assay plate (100 μ L/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TEIRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

Assay Protocol – cAMP

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve 5×10^5 cells/ml (i.e, if collected 5×10^6 TC, $\frac{5 \times 10^6}{5 \times 10^5/ml} = 10$ mL volume)
4. Seed cell suspension into appropriate assay microplate (100 μ L/well for 96-well plate, 25 μ L/well for 384-well plate).
5. When seeding is complete, place the assay plate at room temperature for 30 minutes.
6. Move assay plate to a humidified 37°C 5% CO₂ incubator for 18-24 h.
7. Prepare Assay Buffer (HBSS, 20mM HEPES, pH 7.4)
8. Prepare anti-cAMP-Cryptate and cAMP-D2 conjugate stocks. Prepare stocks according to the Manufacturers Recommendations. Mix gently. Store at 10 to 35 °C after reconstitution. Reagent can only go through one cycle of freeze and thaw.
9. Prepare 1M IBMX Buffer: add 450 μ L of DMSO to 1 vial of 100 mg IBMX powder.
10. Next day, prepare 2mM IBMX Solution: Place the 1M IBMX Buffer and the Assay Buffer in 50-60°C water bath for 10 min to 15 min. Add 10 μ L of 1M IBMX Buffer to 4.990 mL of Assay Buffer. Mix by vortex. Place the 2 mM of IBMX Buffer into 37°C incubator for at least 10 minutes.
11. Prepare 25uL/well of compounds for testing.
12. Remove the cell assay plate from previous day from cell culture incubator. Quickly invert plate on an absorbent pad and blot to remove all media. Add 25 μ L of the 2 mM IBMX Buffer to the plate with the seeded cells. Tap plate gently 3-4 times. Cover plate and incubate inside 37°C incubator, until ready for compound addition.
13. Add 25 μ L compounds, internal control and test sample dilutions to cell assay plate. Cover plate and incubate for 15 to 25 min at 20-25°C
14. Prepare fresh working dilutions of anti-cAMP-Cryptate and cAMP-D2 conjugate in Lysis Buffer according to

- the Manufacturers Recommendations. Protect from light. Do not vortex.
15. It is imperative that detection reagents are added to plate in the following order:
Add 25 μ L cAMP-D2 conjugate/lysis buffer solution to each well of cell assay plate.
Add 25 μ L anti-cAMP-Cryptate/lysis buffer solution to each well of cell assay plate.
 16. Cover with aluminum plate sealer and incubate the cell assay plate 20-25°C, for 60 to 65 min (If available, use gentle plate shaker). Protect from light.
 17. Read plate on a plate reader with 330 nm (excitation) and 665/620 nm (emission).
 18. Calculate Ratio Emission 665/620 nm.

HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, G α 15.

EXOGENOUS GENE EXPRESSION

Full-length human D5 cDNA (Accession Number: NM_000798) and promiscuous G protein are expressed in a bicistronic vector

RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS129M	ChemiScreen™ Receptor D5 Dopamine Receptor Membrane Prep

REFERENCES

1. Centonze D *et al.* (2003) Distinct roles of D₁ and D₅ dopamine receptors in motor activity and striatal synaptic plasticity. *J. Neurosci.* 23: 8506-8512.
2. Hollon TR *et al.* (2002) Mice lacking D₅ 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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