ChemiScreen™ cAMP -OPTIMIZED STABLE CELL LINE HUMAN RECOMBINANT D_{2L} DOPAMINE RECEPTOR

CATALOG NUMBER:	HTS039C2	**QUANTITY:**	2 vials, 1 mL per vial
LOT NUMBER:		**CONCENTRATION:**	2 x 10^6 cells/mL

BACKGROUND:

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). Millipore's cloned human D_{2L}-expressing cell line is made in the CHO host, which supports optimal levels of recombinant D_{2L} expression for robust agonist-induced cAMP signal. Thus, the cell line is an ideal tool for screening for agonists and antagonists at the D_{2L} Receptor.

APPLICATIONS:

Assay for inhibition of forskolin-induced cyclic AMP

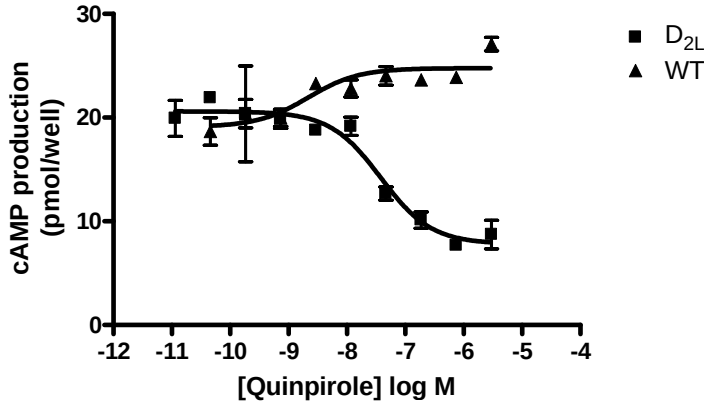

Figure 1. Cyclic AMP assay with D_{2L}-expressing CHO cell line. D_{2L}-expressing CHO cells and wild-type CHO (WT) were preincubated in 1 mM IBMX for 5 min, then exposed to ligand in the presence of 10 μM forskolin for another 15 min at 37ºC. Cells were lysed and cAMP levels were determined with Millipore's cAMP HTS immunoassay kit (catalog # 17-418).

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Table I. Comparison of EC50 values of D_{2L}-expressing CHO cells with values described in the literature.

ligand	assay	potency (nM)	Reference
Quinpirol	cAMP	EC50 = 38	Figure 1
Quinpirol	cAMP	EC50 = 14	Moreland *et al.* (2004)

HOST CELLS: CHO-K1 cells

TRANSFECTION: Plasmid pcDNA3 containing DRD2 long isoform cDNA encoding D_{2L} (Accession Number: NM_ 000795; see CODING SEQUENCE below). The stable clonal cell line was selected by resistance to geneticin, followed by limited dilution cloning. The cell line was tested and found to have equivalent EC50 and signal at 1, 3 and 6 weeks of continuous culture.

PRESENTATION:

Cells are frozen at 2 x 10^6 cells/mL in 90% fetal bovine serum/10% DMSO. Cell line tests negative for mycoplasma.

STORAGE/HANDLING:

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. Transfer contents of the vial to a T75 flask containing growth media. Place the flask in a humidified incubator at 37ºC with 5% CO_2.
3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca^{++} and Mg^{++} (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37ºC with 5% CO_2 until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL CHO Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at 5 x 10^6 cells/mL in CHO Freezing Media (cell densities of 2-10 x 10^6 are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at –70°C overnight. Store the vials in liquid nitrogen.
7. Use of cells immediately after thawing is feasible for some cell lines and is being

further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays. Cells should be resuspended in CHO Plating Media for plating for calcium assay.

MEDIA:

CHO Growth Media:
F12-K containing 2 mM L-glutamine (Invitrogen 21127)
10% heat-inactivated FBS
1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)
250μg/mL Genetecin/G-418

CHO Plating Media:
F12-K containing 2 mM L-glutamine (Invitrogen 21127)
10% heat-inactivated FBS
1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)

CHO Freezing Media:
90% heat-inactivated FBS
10% DMSO (cell culture grade)

EXAMPLE CYCLIC AMP ASSAY CONDITIONS:

1. Cells propagated for screening should be maintained and seeded at less than 90% confluency. Trypsinize cells as above and seed cells in 96-well tissue culture plate at 50,000 cells/well in CHO Plating Media. Incubate plate overnight in a humidified incubator at 37ºC with 5% CO_2.
2. Remove media from the cells and add 50ul/well of cAMP assay buffer (HBSS containing calcium and magnesium, with 10 mM HEPES) containing 2mM IBMX. Incubate cells in a humidified 37°C/5% CO_2 incubator for 5 min.
3. Add 50ul/well of cAMP assay buffer by itself or containing 2x final concentration of desired concentration of control or testing compounds and 20 μM forskolin. Incubate cells in a humidified 37°C/5% CO_2 incubator for 15 min.
4. Terminate the reaction by adding 100ul/well of lysis buffer from cAMP HTS immunoassay kit (Millipore 17-418), and perform cAMP quantitation according to the kit instructions.

REFERENCES:

Grandy DK *et al.* (1989) Cloning of the cDNA and gene for a human D_2 dopamine receptor. *Proc. Natl. Acad. Sci. USA* 86:9762-6.

Giros B *et al.* (1989) Alternative splicing directs the expression of two D_2 dopamine receptor isoforms. *Nature* 342:923-6.

Missale C *et al.* (1998) Dopamine receptors: from structure to function. *Physiol. Rev.* 78: 189-225.

Moreland RB *et al*. (2004) Comparative pharmacology of human dopamine D_2-like receptor stable cell lines coupled to calcium flux through $G\alpha_{qo5}$. *Biochem. Pharmacol.* 68:

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761-772.

CODING SEQUENCE:

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   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   *
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