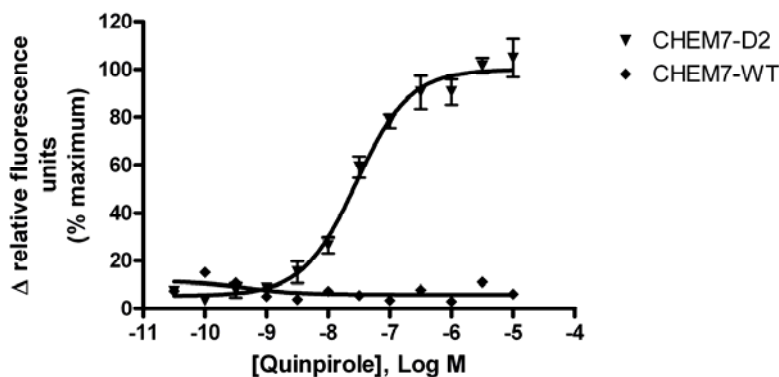


**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT D<sub>2</sub> DOPAMINE RECEPTOR**

<b>CATALOG NUMBER:</b>	HTS039C	<b>QUANTITY:</b>	2 vials, 1 mL per vial
<b>LOT NUMBER:</b>		<b>CONCENTRATION:</b>	2 x 10 <sup>6</sup> cells/mL

**BACKGROUND:** 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 D<sub>1</sub> and D<sub>5</sub> subtypes couple to G<sub>s</sub> to increase intracellular cAMP, whereas the D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub> subtypes couple to G<sub>i</sub> to reduce cAMP (Missale *et al.*, 1998). The D<sub>2</sub> dopamine receptors have been of particular clinical interest due to their regulation of prolactin secretion and their affinity for antipsychotic drugs. The D<sub>2</sub> receptor exists as two alternatively spliced isoforms differing in the insertion of a stretch of 29 amino acids in the third intracellular loop (D<sub>2S</sub> and D<sub>2L</sub>) (Giros *et al.*, 1989; Grandy *et al.*, 1989). Chemicon's cloned human D<sub>2L</sub>-expressing cell line is made in the Chem-7 host, which supports high levels of recombinant D<sub>2</sub> expression on the cell surface and contains high levels of the promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between D<sub>2</sub> and its ligands.

**APPLICATIONS:** Calcium flux assay, ligand binding assays

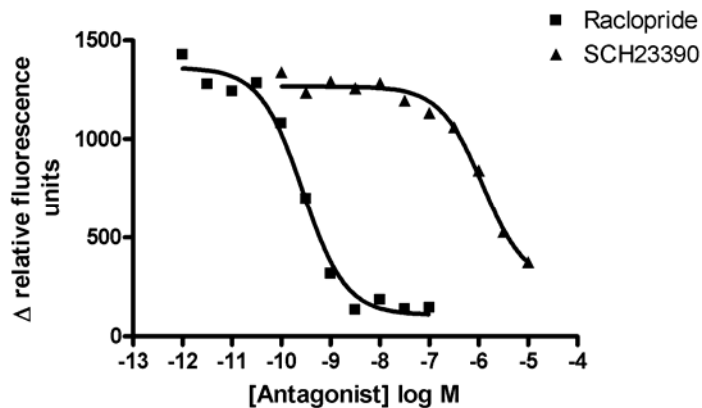


**Figure 1.** Calcium flux in D<sub>2</sub>-expressing Chem-7 cell line induced by Quinpirole. D<sub>2</sub>-expressing Chem-7 cells and Wild-Type Chem-7 cells were loaded with Fluo-4 NW and calcium flux in response to Quinpirole (10<sup>-5</sup> to 10<sup>-10.5</sup> M) was determined in triplicate on a Molecular Devices FLIPR<sup>TETRA™</sup>.

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**Figure 2.** Assay for antagonist activity at D2 by calcium flux assay. D2-expressing Chem-7 cells were loaded with Fluo-4. Raclopride (D2-like selective) and SCH23390 (D1-like selective) were added to the cells at the final concentration indicated, and incubated for 10 min at 37°C. Calcium flux in response to quinpirole (60 nM) was determined in triplicate on a Molecular Devices FLIPRETETRA.

SPECIFICATIONS: EC50 for calcium mobilization by Dopamine: ~ 76 nM  
 EC50 for calcium mobilization by Quinpirole: ~ 29 nM  
 IC50 for Raclopride with 2x EC50 Dopamine: 0.22 nM  
 IC50 for SCH23390 with 2x EC50 Dopamine: 831 nM

HOST CELLS: Chem-7, an adherent cell line expressing a promiscuous G-protein.

TRANSFECTION: Full-length human DRD2 cDNA encoding D<sub>2</sub> (Accession Number: NM\_000795)

**PRESENTATION:**

Cells are frozen at 2 x 10<sup>6</sup> cells/mL in F-12K Nutrient Mixture, Kaighn's Modification /20% heat inactivated 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. Maintain frozen in liquid nitrogen for up to 5 years.
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. Transfer contents of the vial to a T75 flask containing growth media. Place the flask in a humidified incubator at 37°C with 5% CO<sub>2</sub>.
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<sup>++</sup> and Mg<sup>++</sup> (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37°C with 5% CO<sub>2</sub> 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 Chem-1 Growth Media per 1 mL trypsin.

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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  $5 \times 10^6$  cells/mL in Freezing Media (cell densities of  $2-10 \times 10^6$  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  $-70^\circ\text{C}$  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 Plating Media for plating for calcium assay.

## MEDIA:

### Chem-7 Growth Media:

F-12K Nutrient Mixture, Kaighn's Modification containing 2 mM L-glutamine (Invitrogen 21127)  
10% heat-inactivated FBS  
100 U/mL Pen-Strep (from 100x stock, Millipore TMS-AB2-C)  
G-418 (250ug/mL) (from 50 mg/mL stock, Invitrogen 10131-027)  
Zeocin (200 ug/mL) (from 100 mg/mL stock, Invitrogen 45-0430)

### Chem-7 Plating Media:

F-12K Nutrient Mixture, Kaighn's Modification  
10% heat-inactivated FBS  
1x Pen-Strep

### Chem-7 Freezing Media:

F-12K Nutrient Mixture, Kaighn's Modification  
20% heat-inactivated FBS  
1x Pen-Strep  
10% DMSO (cell culture grade)

## REFERENCES:

- 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.
- Giros B *et al.* (1989) Alternative splicing directs the expression of two D2 dopamine receptor isoforms. *Nature.* 342:923-6.
- Missale C *et al.* (1998) Dopamine receptors: from structure to function. *Physiol. Rev.* 78: 189-225.

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