

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT  $\alpha_{1A}$  ADRENOCEPTOR**

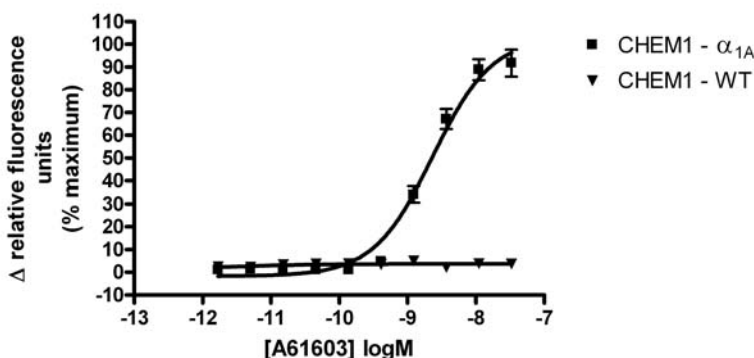
<b>CATALOG NUMBER:</b>	HTS087C	<b>QUANTITY:</b>	2 vials, 1 mL per vial
<b>LOT NUMBER:</b>		<b>CONCENTRATION:</b>	$2 \times 10^6$ cells/mL

**BACKGROUND:**

The endogenous catecholamines epinephrine and norepinephrine have profound effects on smooth muscle activity, cardiac function, carbohydrate and fat metabolism, hormone secretion, neurotransmitter release, and central nervous system actions. These activities are mediated by GPCRs belonging to two subfamilies, the  $\alpha$ - and  $\beta$ -adrenoceptors (Bylund *et al.*, 1994). The three members of the  $\alpha_1$  subclass of adrenoceptors,  $\alpha_{1A}$ ,  $\alpha_{1B}$  and  $\alpha_{1D}$ , couple to  $G_q$ , and promote contraction of vascular and urinary tract smooth muscle, relaxation of intestinal smooth muscle, increased contractile force in the heart, and glycogenolysis and gluconeogenesis in the liver. The different subtypes have overlapping distributions and variably contribute to these effects depending on species and tissue; the  $\alpha_{1A}$  subtype plays a prominent role in urogenital smooth muscle contraction and renal artery contraction (Hrometz *et al.*, 1999; Ruffolo and Hieble, 1999). Activation of  $\alpha_1$  adrenoceptors also influences cell proliferation;  $\alpha_{1A}$  inhibits cell growth by arresting progression at the  $G_1/S$  transition (Shibata *et al.*, 2003). The  $\alpha_{1A}$  subtype undergoes alternative splicing to generate four variants that differ at their C-termini, although these variants appear to be functionally identical (Chang *et al.*, 1998). Chemicon's cloned human  $\alpha_{1A}$ -expressing cell line is made in the Chem-1 host, which supports high levels of recombinant  $\alpha_{1A}$  expression on the cell surface and contains high levels of the promiscuous G protein  $G\alpha_{15}$  to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between  $\alpha_{1A}$  and its ligands.

**APPLICATIONS:**

Calcium flux assay, ligand binding assays



**Figure 1.** Calcium flux in  $\alpha_{1A}$ -expressing Chem-1 cell line induced by A61603.  $\alpha_{1A}$ -expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 NW and calcium flux in response to recombinant human A61603 ( $10^{-7.5}$  to  $10^{-12}$  M) was determined in triplicate on a Molecular Devices FLIPR TETRA.

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SPECIFICATIONS: EC50 for calcium mobilization by A61603: ~ 2.3 nM

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, G $\alpha$ 15.

TRANSFECTION: Full-length human ADRA1A transcript variant 1 cDNA encoding  $\alpha_{1A}$  (Accession Number: NM\_000680)

GROWTH MEDIA: DMEM containing 4.5 g/L glucose/10% heat inactivated fetal bovine serum/1x nonessential amino acids/10 mM HEPES/0.25 mg/ml Geneticin (G418)/100 U/ml each penicillin and streptomycin

**PRESENTATION:**

Cells are frozen at  $2 \times 10^6$  cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. Cell line tests negative for mycoplasma.

**STORAGE/HANDLING:**

Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years. 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 20 mL growth media, and place in a humidified 37°C incubator with 5% CO<sub>2</sub>. After 8-24 h, cells will adhere to the plate, at which time the media should be replaced to remove residual DMSO. Cells are passaged by washing with Ca<sup>++</sup> and Mg<sup>++</sup>-free HBSS (10 mL/T75), incubating with 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) for 5-10 minutes at 37°C, and rapping the side of the flask to dislodge the cells. Neutralize the trypsin by addition of 4 volumes growth media. Cells are typically passaged 1:10 with every 3-4 days, and should be passaged at least once after thawing prior to use in calcium flux assays.

**REFERENCES:**

Bylund DB *et al.* (1994). IV. International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.* 46: 121-136.

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Hrometz SL *et al.* (1999) Expression of multiple alpha1-adrenoceptors on vascular smooth muscle: correlation with the regulation of contraction. *J. Pharmacol. Exp. Ther.* 290(1):452-63.

Ruffolo JR RR and Hieble JP (1999) Adrenoceptor pharmacology: urogenital applications. *Eur. Urol.* 36 (suppl. 1): 17-22.

Shibata K *et al.* (2003)  $\alpha_1$ -Adrenergic receptor subtypes differentially control the cell cycle of transfected CHO cells through a cAMP-dependent mechanism involving p27<sup>Kip1</sup>. *J. Biol. Chem.* 278: 672-678.

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