

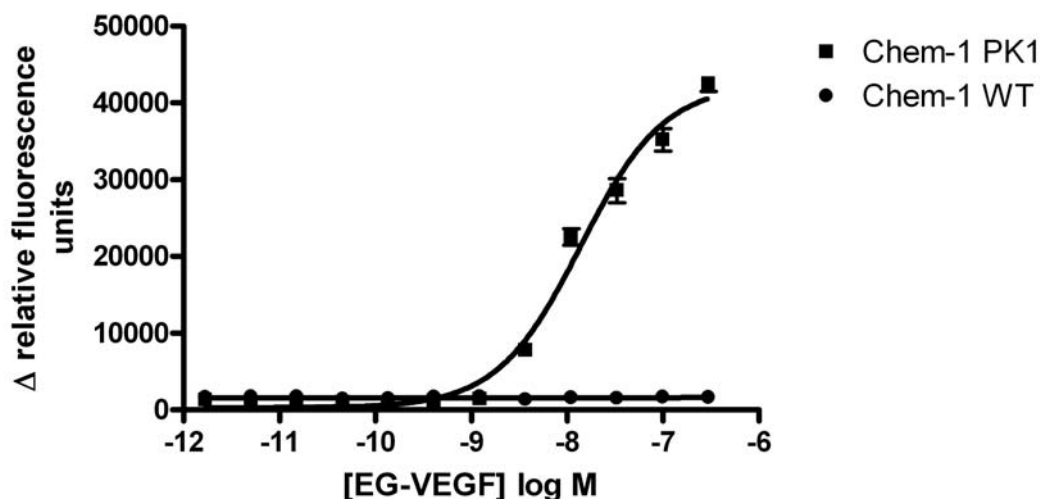
**ChemiScreen<sup>™</sup> CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT PK1 PROKINETICIN RECEPTOR 1**

**CATALOG NUMBER:** HTS074C **QUANTITY:** 2 vials, 1 mL per vial

**LOT NUMBER:** **CONCENTRATION:**  $2 \times 10^6$  cells/mL

**BACKGROUND:** Prokineticins, also known as endocrine gland vascular endothelial growth factors (EG-VEGF), are two ~10 kD secreted proteins originally described to mediate angiogenesis and gastrointestinal smooth muscle contraction, and (Li et al., 2001; LeCouter et al., 2003). Subsequently, prokineticins have been found to mediate central nervous system functions including circadian rhythms and olfactory bulb development (Cheng et al., 2002; Ng et al., 2005). Two Gq-coupled receptors, PK1 and PK2 (also known as GPR73a and GPR73b), mediate cellular responses to prokineticins (Lin et al., 2002). Chemicon's cloned human PK1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant PK1 expression on the cell surface and contains high levels of the promiscuous G protein  $G_{\alpha 15}$  to enhance coupling of the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between PK1 and its ligands.

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



**Figure 1.** Calcium flux in PK1-expressing Chem-1 cell line induced by EG-VEGF. PK1-expressing Chem-1 (Chem-1 PK1) and Wild-Type Chem-1 cells (Chem-1 WT, Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to EG-VEGF ( $10^{-5.5}$  to  $10^{-12}$  M) was determined in triplicate on a Molecular Devices Flex Station. An increase in fluorescence units of greater than 40,000 was obtained with EG-VEGF.

**SPECIFICATIONS:** EC50 for calcium mobilization by EG-VEGF: ~ 14 nM

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

**TRANSFECTION:** Full-length human GPR73 cDNA, encoding PK1 (Accession Number: NM\_138964)

**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:**

Cheng MY *et al.* (2002) Prokineticin 2 transmits the behavioral circadian rhythm of the suprachiasmatic nucleus. *Nature* 417: 405-10.

LeCouter J *et al.* (2003) Endocrine gland-derived VEGF and the emerging hypothesis of organ-specific regulation of angiogenesis. *Nat. Med.* 8: 913-7.

Li M *et al.* (2001) Identification of two prokineticin cDNAs: recombinant proteins potently contract gastrointestinal smooth muscle. *Mol. Pharmacol.* 59: 692-8.

Lin DC *et al.* (2002) Identification and molecular characterization of two closely related G protein-coupled receptors activated by prokineticins/endocrine gland vascular endothelial growth factor. *J. Biol. Chem.* 277: 19276-80.

Ng KL *et al.* (2005) Dependence of olfactory bulb neurogenesis on prokineticin 2 signaling. *Science* 308: 1923-7.

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HUMAN RECOMBINANT PK1 PROKINETICIN RECEPTOR 1**

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