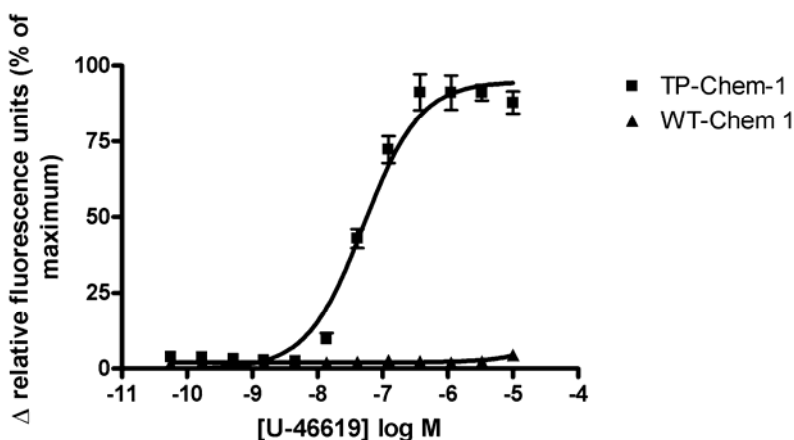


**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT TP PROSTANOID RECEPTOR**

<b>CATALOG NUMBER:</b>	HTS081C	<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:** TP receptors (Thromboxane A<sub>2</sub> receptors) are widely distributed among different organ systems and have been localized on both cell membranes and intracellular structures. TP receptors belong to G protein-coupled receptor family (Hirata *et al.*, 1991). Activation of TP receptors induces platelet aggregation, vascular and respiratory smooth muscle constriction, and enhances mitogenic responses of vascular smooth muscle cells that are stimulated by growth factors (Ali *et al.*, 1993; Hanasaki *et al.*, 1990). The human TP receptor is encoded by a single gene that is alternatively spliced at the carboxyl terminus, resulting in two isoforms, TP $\alpha$  (343 residues) and TP $\beta$  (407 residues). Both isoforms couple to Gq pathway, but couple oppositely to adenylate cyclase. The cDNA encoding human TP $\alpha$  has been stably expressed in the Chem-1 host, which supports high levels of recombinant receptor 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 TP and its ligands.

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



**Figure 1.** Calcium flux in TP-expressing Chem-1 cell line induced by U46619. TP-expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with Fluo-4 and calcium flux in response to U46619 (10<sup>-5</sup> to 10<sup>-9</sup> M) was determined in triplicate on a Molecular Devices Flex Station.

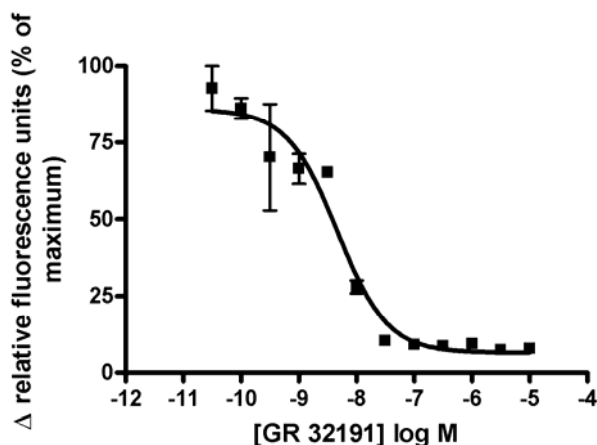


Figure 2. Inhibition of U-46619-induced calcium signalling in TP-expressing Chem-1 cells by GR 32191. TP-expressing Chem-1 cells were loaded with Fluo-4 and preincubated with the indicated concentration of GR 32191 for 10 min. Calcium flux in response to 88 nM U-46619 was determined on a Molecular Devices FLIPR<sup>TETRA</sup><sup>™</sup>. An IC50 of 4.6 nM for GR 32191 was obtained.

SPECIFICATIONS: EC50 for calcium mobilization by U-46619: ~ 44.2 nM  
IC50 for GR 32191: 3-9 nM  
Signal/noise at agonist E<sub>max</sub>: 534

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

TRANSFECTION: Full-length human TBXA2R cDNA encoding the TP short form (TP $\alpha$ ) (Accession Number: NM\_001060)

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 x 10<sup>6</sup> 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:**

Ali S *et al.* (1993) Thromboxane A<sub>2</sub> stimulates vascular smooth muscle hypertrophy by unregulating the synthesis and release of endogenous basic fibroblast growth factor. *J Biol. Chem.* 268:17397–17403.

Hanasaki K *et al.* (1989) Biochemical characterization and comparison of rat thromboxane A<sub>2</sub>/prostaglandin H<sub>2</sub> receptors in platelets and cultures aortic smooth muscle cells. *Biochem. Pharmacol.* 38:2967–2976.

Hirata M *et al.* (1991) Cloning and expression of the cDNA for a human thromboxane A<sub>2</sub> receptor gene. *Nature* 349: 617-620.

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HUMAN RECOMBINANT TP PROSTANOID RECEPTOR**

**Product No. HTS081C**

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