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

CATALOG NUMBER:	HTS081AG	**QUANTITY:**	2 vials, 1 mL per vial
LOT NUMBER:	R0711E0007	**CONCENTRATION:**	2 x 10^6 cells/mL

BACKGROUND: TP receptors (Thromboxane A2 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, TPα (343 residues) and TPβ (407 residues). Both isoforms couple to Gq pathway, but couple oppositely to adenylate cyclase. The cDNA encoding human TPα has been stably expressed in the Chem-1 host which stably expresses a mitochondrially targeted glowing mutant form of aequorin. This glow variant of aequorin has a unique long-lasting luminescent profile compared to flash variants. Thus, the cell line is an ideal tool for screening for agonists and antagonists at TP.

APPLICATIONS: Luminescent and fluorescent calcium flux assays, ligand binding assays

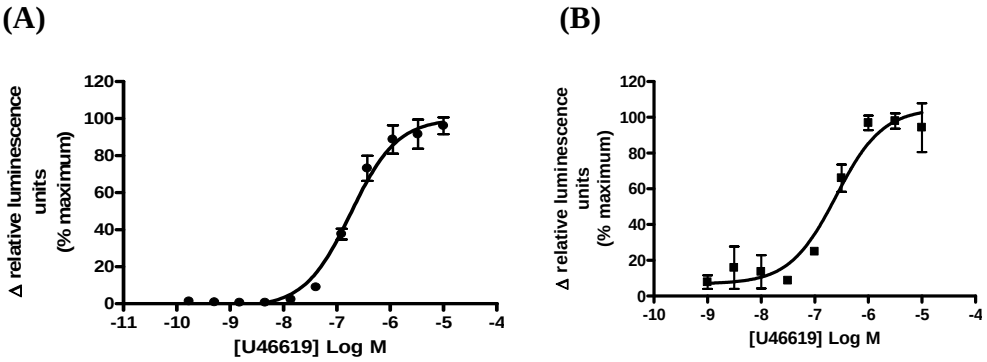

Figure 1. Ligand-induced calcium flux in Glow Aequorin Chem-1 cell line stably transfected with TP. Glow Aequorin Chem-1 stably co-expressing TP were loaded with 5 μM coelenterazine for 3 h at room temperature. (A) Luminescence in response to U46619 was determined in quadruplicate in a 384 well plate with a FLIPR^{TETRA} with aequorin option (Molecular Devices, now part of MDS Analytical Technologies). Data were collected for area under curve for 240 sec. (B) Luminescence in response to U46619 was determined in duplicate in a 96 well plate with a Perkin Elmer Wallac Victor2. Data were collected for area under curve for 20 sec.

SPECIFICATIONS: EC50 for calcium mobilization by U46619:
~182.7 nM (FLIPR^{TETRA} system) ~ 250.5 nM (Wallac Victor2)

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

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TRANSFECTION: Full-length human TBXA2R cDNA encoding the TP short form (TPα) (Accession Number: NM_001060)

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. Maintain frozen in liquid nitrogen for up to 5 years.
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 (2-4 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Chem1 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 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.

MEDIA:

Chem-1 Aequorin Growth Media:
DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
100 U/mL Pen-Strep (from 100x stock, Millipore TMS-AB2-C)
250µg/mL Genetecin/G-418
250µg/mL Hygromycin

Chem-1 Aequorin Plating Media:
DMEM with 4.5 g/L glucose and 4 mM glutamine
10% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep

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

RECOMMENDED ASSAY CONDITIONS:

1. Seed cells in 96-well white plate (top-read instruments) or opaque-walled, clear bottom plate (bottom-read instruments) overnight at 50,000 cells/well in Chem-1 Aequorin Plating Media.
2. Wash cells once (200 µl/well) with Wash Buffer (HBSS with Ca^{++} and Mg^{++} containing 10 mM HEPES) before loading with 5µM of coelenterazine (Millipore ES016) in wash buffer at room temperature for 3 hours.

   Note: *Luminescence activity has been determined to be optimal at room temperature. Incubation at 37 °C will result in substantially reduced signals.*

3. After loading, wash cells once with Wash Buffer (200 µl/well) prior to addition of ligands.

REFERENCE:

Ali S *et al.* (1993) Thromboxane A2 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 A2/prostaglandin H2 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_2 receptor gene. *Nature* 349: 617-620.

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