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PRODUCT DATASHEET

ChemiScreen™ PAR2 Protease-Activated Membrane Preparation

CATALOG NUMBER: HTS037M **QUANTITY:** 200 units

LOT NUMBER: **VOLUME/CONCENTRATION:** 1 mL, 1 mg/mL

BACKGROUND:

The protease-activated receptor family of GPCRs has a unique mechanism of activation, in which protease cleaves a prodomain to reveal a peptide sequence that functions as a tethered ligand to activate the receptor (Macfarlane *et al*., 2001). PAR2 is specifically activated by trypsin and mast cell tryptase, and can also be activated by free peptide analogs of the tethered ligand, such as SLIGRL and furoyl-LIGRLO (Coelho *et al*., 2003; Kawabata *et al*., 2004). PAR2 is expressed in endothelium, gastrointestinal epithelium, macrophages, eosinophils and nociceptive afferent neurons. Activation of PAR2 in these cells and tissues promotes vasodilation, inflammation, allergy, hyperalgesia and intestinal permeability. Therefore, PAR2 is regarded as an attractive therapeutic target for colitis, asthma, myocardial ischemia/reperfusion injury, and pain (Cocks *et al*., 1999; Hansen *et al*., 2005; Lindner *et al*., 2000; McLean *et al*., 2002; Vergnolle *et al*., 2001). PAR2 membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression; thus, they are ideal HTS tools for screening of antagonists of PAR2 interactions with its ligands. The membrane preparations exhibit an EC50 of 1.9 μM for furoyl-LIGRLO in a GTPγS binding assay.

APPLICATIONS: GTPγS Binding

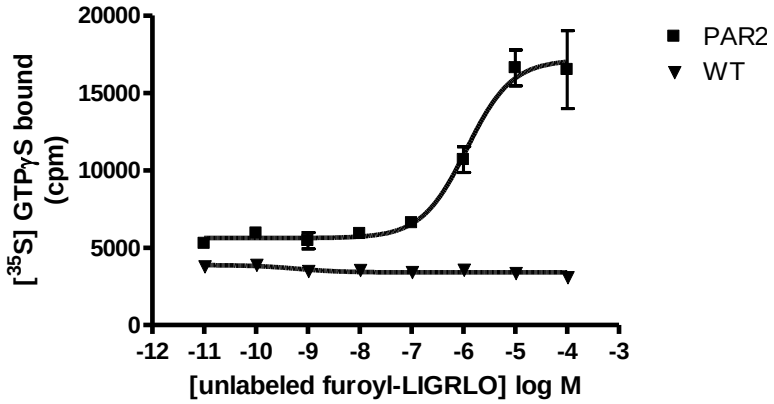

Figure 1. Binding of [^{35}S]GTPγS to PAR2 membrane preparation. 5 μg/well PAR2 Membrane Preparation (catalog # HTS037M) and Wild-Type Chem-1 Membrane Preparation (catalog # HTS000MC1) was incubated with 0.1 nM [^{35}S]-GTPγS and increasing amounts of unlabeled furoyl-LIGRLO. Bound radioactivity was determined by filtration and scintillation counting.

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SPECIFICATIONS: 1 unit = 5 µg
EC50 for furoyl-LIGRLO: ~3.8 µM in a GTPγS binding assay

Species: Human PAR2 (Accession number AY336105)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous PAR2 expression.

RECOMMENDED ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [^{35}S]-GTPγS (final concentration of 0.1 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM $MgCl_2$/0.5 µM GDP in a nonbinding 96-well plate. Unlabeled furoyl-LIGRLO is added to the final concentration indicated in Figure 1 (final volume 100 µL), and incubated for 30 min at 30°C. The binding reaction is transferred to an FB filter plate (EMD Millipore MAHF B1H) prewetted with water, and washed 3 times (1 mL per well per wash) with cold 10 mM sodium phosphate, pH 7.4. The plate is dried and counted.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 1000 cpm specific furoyl-LIGRLO-stimulated [^{35}S]-GTPγS binding.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.

Packaging method: Membrane proteins were adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING: Store at –70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

1. Cocks TM et al. (1999) A protective role for protease-activated receptors in the airways. Nature 398: 156-60.
2. Coelho AM *et al.* (2003) Proteinase-activated receptor-2: physiological and pathophysiological roles. *Curr. Med. Chem. Cardiovasc. Hematol. Agents.* 1: 61-72.
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8. Vergnolle N *et al.* (2001) Proteinase-activated receptor-2 and hyperalgesia: A novel pain pathway. *Nat. Med.* 7: 821-6.

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