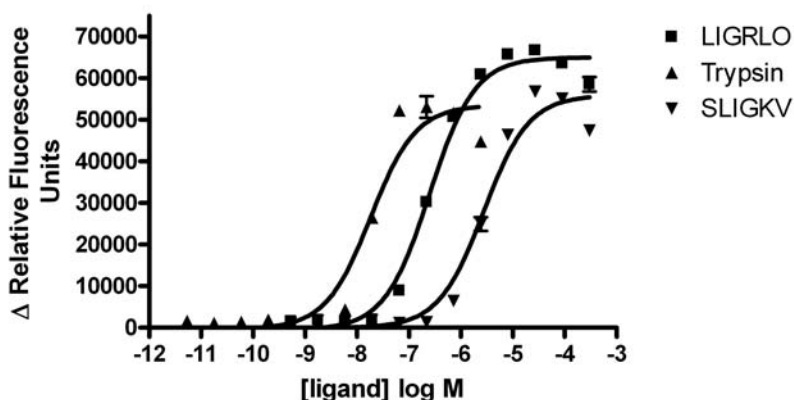


**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT PAR2 PROTEINASE-ACTIVATED RECEPTOR**

<b>CATALOG NUMBER:</b>	HTS037C	<b>QUANTITY:</b>	2 vials, 1 mL each
<b>LOT NUMBER:</b>		<b>CONCENTRATION:</b>	2 x 10 <sup>6</sup> cells/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). Chemicon's cloned human PAR2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant PAR2 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 PAR2 and its ligands.

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



**Figure 1.** Calcium flux in PAR2-expressing Chem-1 cell line. PAR2-expressing Chem-1 cells were loaded with Fluo-4 and calcium flux in response to Trypsin, SLIGKV, and furoyl-LIGRLO ( $10^{-3}$  to  $10^{-11}$  M) was determined in duplicate on a Molecular Devices Flex Station. A signal:background ratio of greater than 20 was obtained.

**SPECIFICATIONS:** EC<sub>50</sub> for calcium mobilization by 2-furoyl-LIGRLO: ~ 250 nM  
EC<sub>50</sub> for calcium mobilization by Trypsin: ~ 18 nM  
EC<sub>50</sub> for calcium mobilization by SLIGKV: ~ 2  $\mu$ M

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

TRANSFECTION: Full-length human PAR2 cDNA (Accession Number: AY336105)

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.

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