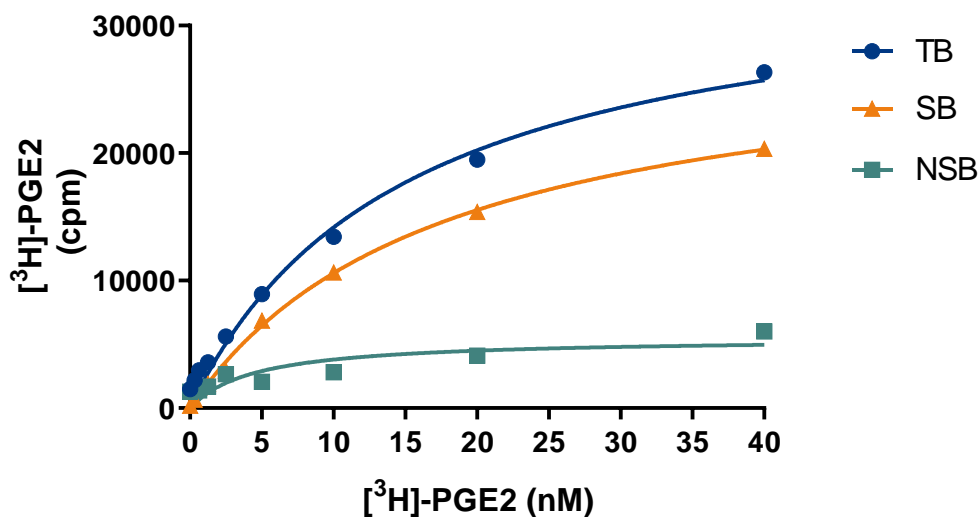


**PRODUCT DATASHEET**
**ChemiScreen™ EP<sub>1</sub> Prostanoid Membrane Preparation**

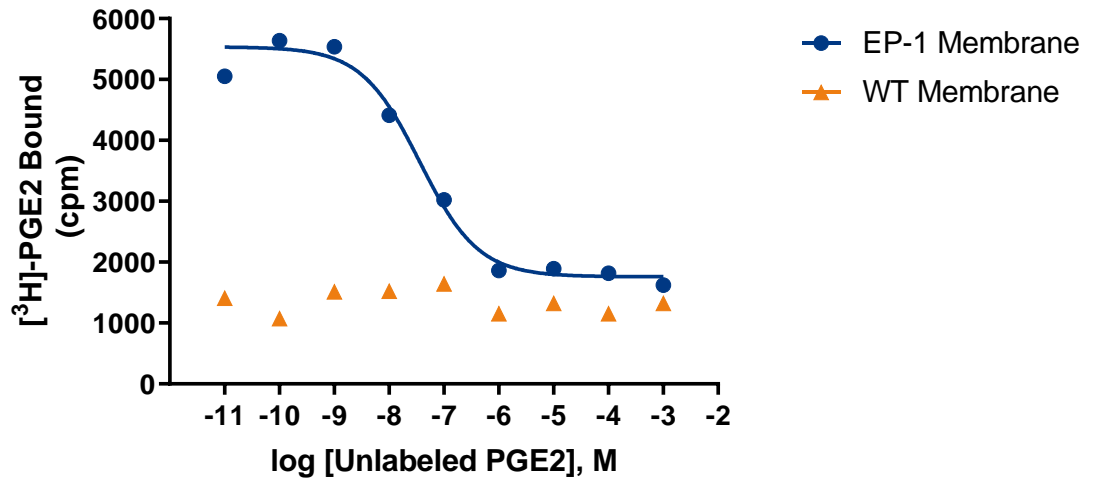
<b>CATALOG NUMBER:</b>	HTS099M	<b>QUANTITY:</b>	200 units
<b>LOT NUMBER:</b>	SC20190314	<b>VOLUME/CONCENTRATION:</b>	1 mL, 2 mg/mL

**BACKGROUND:** Prostanoids are a series of arachidonic acid metabolites produced by the action of cyclooxygenase and subsequently by isomerases and synthases. Cells rapidly secrete prostanoids after synthesis, whereupon the prostanoids bind to a family of 8 GPCRs to exert their biological effects (Narumiya and FitzGerald, 2001). The prostaglandin PGE<sub>2</sub> causes pain, vasodilation, immunosuppression of T cells, bone resorption and promotion of carcinogenesis. Four related GPCRs, EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub> and EP<sub>4</sub>, each bind to PGE<sub>2</sub>, but the different G protein coupling status of each receptor leads to distinct biological effects; EP<sub>1</sub> couples primarily to G<sub>q</sub> to mobilize intracellular calcium. EP<sub>1</sub> appears to mediate the effects of PGE<sub>2</sub> in promoting formation of precancerous lesions in animal models of colon cancer (Watanabe *et al.*, 1999). In addition, EP<sub>1</sub> has an inhibitory effect on stress-induced aggressive and risk-taking behaviors in mice (Matsuoka *et al.*, 2005). EP<sub>1</sub> 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 agonists and antagonists of EP<sub>1</sub>. The membrane preparations exhibit a K<sub>d</sub> of 17.58 nM for [<sup>3</sup>H]-PGE<sub>2</sub>. With 40 nM [<sup>3</sup>H]-PGE<sub>2</sub>, 10 µg/well EP<sub>1</sub> Membrane Prep typically yields 3-fold signal-to-background ratio.

**APPLICATIONS:** Radioligand binding assay



**Figure 1. Saturation binding for EP<sub>1</sub>.** 10 µg/well EP<sub>1</sub> Membrane Preparation was incubated with increasing amount of [<sup>3</sup>H]-Prostaglandin E<sub>2</sub> in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled PGE<sub>2</sub>. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.



**Figure 2. Competition binding for EP<sub>1</sub>.** EP<sub>1</sub> Membrane Preparation (10 µg/well) was with 10 nM <sup>3</sup>H-Prostaglandin E<sub>2</sub> and increasing concentrations of unlabeled prostaglandin E<sub>2</sub>. 3-fold signal:background was obtained. Sample data from a representative lot.

**SPECIFICATIONS:** 1 unit = 10 µg membrane preparation

B<sub>max</sub>: 15.48 pmol/mg

K<sub>d</sub>: 17.58 nM

Signal:Background: 3-fold

**TRANSFECTION:** Full-length human PTGER1 cDNA encoding EP<sub>1</sub> (Accession Number: NM\_000955)

**HOST CELLS:** Chem-1, an adherent mammalian cell line without detectable endogenous prostaglandin E<sub>1</sub> receptor expression.

**RECOMMENDED ASSAY CONDITIONS:** Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, an FC 96-well harvest plate (EMD Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, was then washed with 10 mM MES, pH 6, 1 mM EDTA, 10 mM MnCl<sub>2</sub>. Binding reaction is transferred to the filter plate and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells are dried and counted.

**Binding buffer:** 10 mM MES, pH 6, 1 mM EDTA, 10 mM MnCl<sub>2</sub>, filtered and stored at 4°C

**Radioligand:** [<sup>3</sup>H]-Prostaglandin E<sub>2</sub>. (Perkin Elmer # NET428)

**Wash Buffer:** 10 mM MES, pH 6, 1mM EDTA, 10 mM MnCl<sub>2</sub>, filtered and stored at 4°C.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield 3-fold signal: background with <sup>3</sup>H-labeled PGE<sub>2</sub>.

**PRESENTATION:**

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

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

**STORAGE/HANDLING:** Store at  $-70^{\circ}\text{C}$ . Product is stable for at least 6 months from the date of receipt when stored as directed. Avoid repeated freeze/thaw cycles.

- REFERENCES:**
1. Matsuoka Y et al. (2005) Prostaglandin E receptor EP<sub>1</sub> controls impulsive behavior under stress. *Proc. Natl. Acad. Sci. USA*. 102: 16066-16071
  2. Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.
  3. Watanabe K et al. (1999) Role of prostaglandin E receptor subtype EP<sub>1</sub> in colon carcinogenesis. *Cancer Res.* 59: 5093-5096.

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