



CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN EP₁ PROSTANOID RECEPTOR

CATALOG NUMBER: HTS099M **QUANTITY:** 200 units
LOT NUMBER: **VOLUME/CONCENTRATION:** 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 PGE₂ causes pain, vasodilation, immunosuppression of T cells, bone resorption and promotion of carcinogenesis. Four related GPCRs, EP₁, EP₂, EP₃ and EP₄, each bind to PGE₂, but the different G protein coupling status of each receptor leads to distinct biological effects; EP₁ couples primarily to G_i to mobilize intracellular calcium. EP₁ appears to mediate the effects of PGE₂ in promoting formation of precancerous lesions in animal models of colon cancer (Watanabe *et al.*, 1999). In addition, EP₁ has an inhibitory effect on stress-induced aggressive and risk-taking behaviors in mice (Matsuoka *et al.*, 2005). Millipore's EP₁ 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 EP₁. The membrane preparations exhibit a K_d of 14.5 nM for [³H]-PGE₂. With 40 nM [³H]-PGE₂, 10 µg/well EP₁ Membrane Prep typically yields greater than 3-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

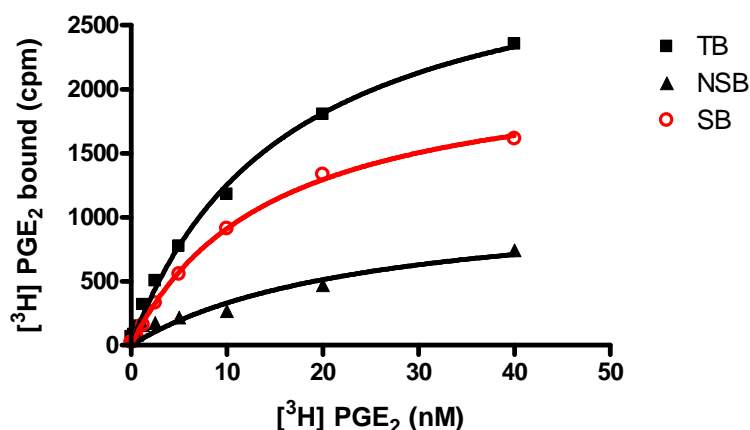


Figure 1. Saturation binding for EP₁. 10 µg/well EP₁ Membrane Preparation was incubated with increasing amount of [³H]-PGE₂ in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled PGE₂. Specific binding (SB) was determined by subtracting NSB from TB.

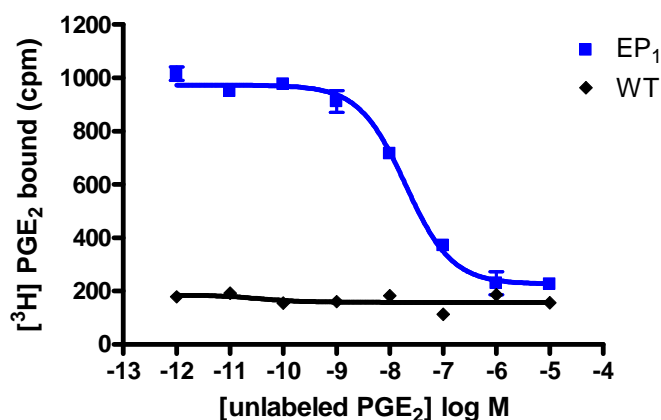


Figure 2. Competition binding for EP₁. 10µg/well EP₁ Membrane Preparation and wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 10 nM ³H-labeled PGE₂ and increasing concentrations of unlabeled PGE₂. More than 3-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with EP₁ membrane prep.

	10 µg/well
Signal:background	4.3
Specific binding (cpm)	724

SPECIFICATIONS: 1 unit = 10 µg membrane preparation
Bmax: 4.6 pmol/mg
K_d: 14.5 nM

TRANSFECTION: Full-length human PTGER1 cDNA encoding EP₁ (Accession Number: NM_000955)

HOST CELLS: Chem-1, an adherent mammalian cell line without detectable endogenous prostaglandin E₂ 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 (Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 10mM MES, pH 6, 1mM EDTA, 10mM MnCl₂. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash



Buffer. The plate is dried and counted.

Binding buffer: 10mM MES, pH 6, 1mM EDTA, 10mM MnCl₂, filtered and stored at 4°C
Radioligand: [³H]- PGE₂. (Perkin Elmer # NET-428)

Wash Buffer: 10mM MES, pH 6, 1mM EDTA, 10mM MnCl₂, 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 greater than 3-fold signal: background with ³H labeled PGE₂.

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°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

Matsuoka Y et al. (2005) Prostaglandin E receptor EP₁ controls impulsive behavior under stress. *Proc. Natl. Acad. Sci. USA*. 102: 16066-16071.

Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.

Watanabe K *et al.* (1999) Role of prostaglandin E receptor subtype EP₁ in colon carcinogenesis. *Cancer Res.* 59: 5093-5096.

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.

**FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC
PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION**

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