

PRODUCT DATASHEET
ChemiScreen™ EP4 Prostanoid Membrane Preparation

CATALOG NUMBER:	HTS142M	QUANTITY:	200 units
LOT NUMBER:	21C3003	VOLUME/CONCENTRATION:	1 mL, 2 mg/mL

BACKGROUND: Prostanoids are a series of arachidonic acid metabolites produced by the action of cyclooxygenase and further modified 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 remodeling 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. EP₄ couples primarily to G_s to increase intracellular cAMP levels. During neonatal development, EP₄ participates in closure of the ductus arteriosus, a process required for switching circulation from the placenta to the lungs (Nguyen *et al.*, 1997). In addition, EP₄ mediates PGE₂-induced bone formation by promoting osteoblastogenesis, and selective EP₄ agonists are being evaluated as potential treatments for osteoporosis (Yoshida *et al.*, 2002). EP₄ 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 EP₄ interactions with prostaglandin E₂. The membrane preparations exhibit a K_d of 1.75 nM for [³H]-Prostaglandin E₂.

APPLICATIONS: Radioligand binding assay

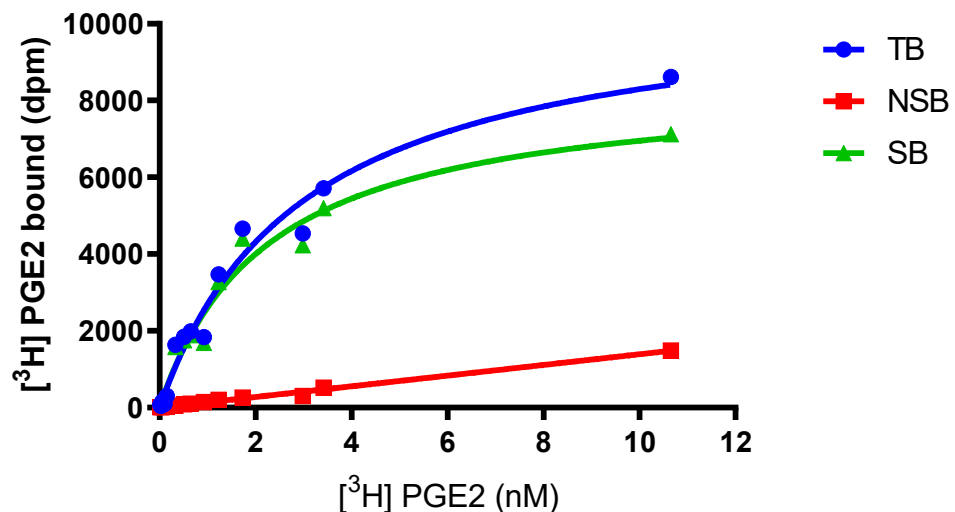


Figure 1. Saturation Binding for EP₄. 10 μg/well EP₄ Membrane Preparation was incubated with increasing amount of [³H]-Prostaglandin E₂ in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled prostaglandin E₂. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample lot.

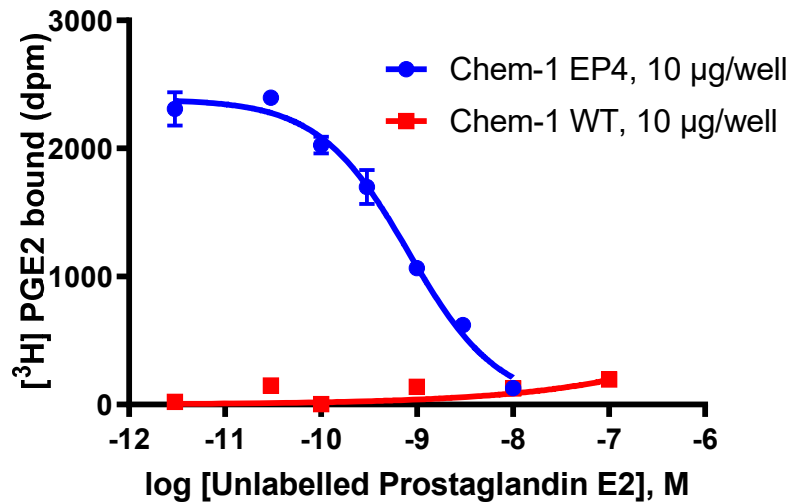


Figure 2. Competition Binding for EP4. EP₄ Membrane Preparation (10 µg/well) was incubated with 3 nM [³H]-Prostaglandin E₂ and increasing concentrations of unlabeled prostaglandin E₂. The data are from a representative sample lot.

SPECIFICATIONS: 1 unit = 10 µg
 B_{max} for [³H]-PGE₂ binding: 1.9 pmol/mg
 K_d for [³H]-PGE₂ binding: 2 nM
 Signal:background: >2.5-fold

TRANSFECTION: Human EP4 (Accession number NM_000958)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line without any 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 2 h. Prior to filtration, an FC 96-well harvest plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. The binding reactions are transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells are then dried and counted.

Binding buffer: 50 mM HEPES, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

Radioligand: [³H]-Prostaglandin E₂ (PerkinElmer # NET428)

Wash Buffer: 50 mM HEPES, pH 7.4, 500 mM NaCl, 0.1% BSA, 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 2.5-fold signal:background.

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 1 ml 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. Narumiya S and FitzGerald GA (2001). Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108:25-30.
2. Nguyen M *et al.* (1997). The prostaglandin receptor EP₄ triggers remodelling of the cardiovascular system at birth. *Nature* 390:78-81.
3. Yoshida K *et al.* (2002). Stimulation of bone formation and prevention of bone loss by prostaglandin E EP₄ receptor activation. *Proc. Natl. Acad. Sci. USA* 99:4580-5.

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