

PRODUCT DATASHEET
ChemiScreen™ EP₂ Prostanoid Membrane Preparation

CATALOG NUMBER: HTS185M **QUANTITY:** 200 units
LOT NUMBER: 22A1106 **VOLUME/CONCENTRATION:** 1 mL, 2 mg/mL

BACKGROUND: Prostanoids bind to a family of 8 GPCRs to exert their biological effects (Narumiya and FitzGerald, 2001). The prostanoid 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. EP₂ couples primarily to G_s to increase intracellular cAMP levels. Mice deficient in EP₂ receptor showed impaired ovulation and fertilization, salt-sensitive hypertension (Kennedy *et al.*, 1999). It has been shown that EP₂ receptors are also involved in cancer associated immunodeficiency. Thus, genetic knockout of the EP₂ receptor reduced tumor growth and prolonged survival in mice that had undergone isograft injection of MC26 or Lewis lung carcinoma cells (Yang *et al.*, 2003). EP₂ 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 EP₂. The membrane preparations exhibit a K_d of 8.3 nM for [³H]-PGE₂. With 7.5 nM [³H]-PGE₂, 10 μg/well EP₂ Membrane Prep typically yields ≥ 3-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

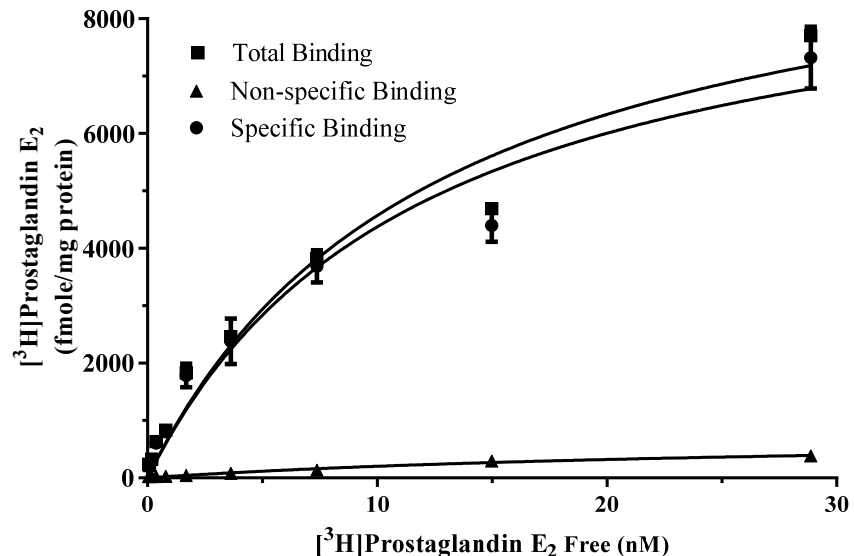


Figure 1. Saturation binding for EP₂. 10 μg/well EP₂ Membrane Preparation was incubated with increasing amount of ³H-labeled PGE₂ in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled PGE₂. Specific binding (SB) was determined by subtracting NSB from TB.

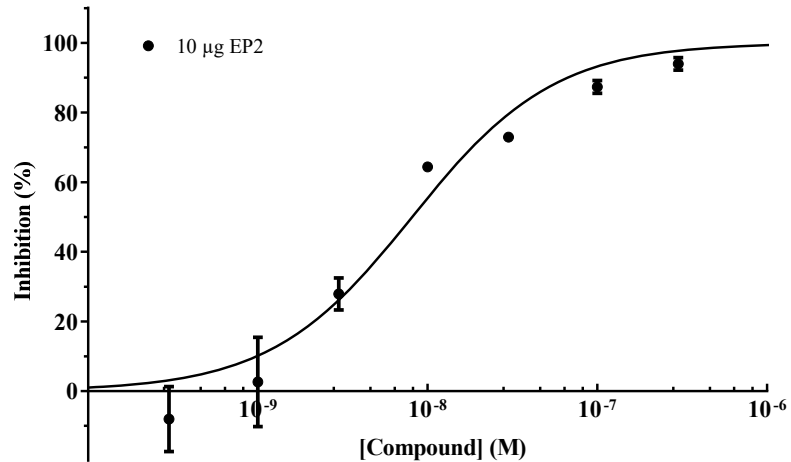


Figure 2. Competition binding for EP₂. 10 µg/well EP₂ Membrane Preparation was incubated in a 96-well plate with 4 nM ³H-labeled PGE₂ and increasing concentrations of unlabeled PGE₂. ≥ 3-fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 10 µg
 B_{max} for [³H]-PGE₂ binding: 9.5 pmol/mg protein
 K_d for [³H]-PGE₂ binding: 12 nM
 Signal:Background: ≥ 3-fold

TRANSFECTION: Full-length human PTGER2 cDNA encoding EP₂ (Accession Number: NM_000956)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous EP₂ 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, then washed with 50mM HEPES, pH 7.4, 500mM NaCl. 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: 50 mM Hepes, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, filtered and stored at 4°C.

Radioligand: [³H]-PGE₂ (Perkin Elmer#: NET-428)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl, 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 at 7.5 nM

PRESENTATION:

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

Packaging method: Membranes protein 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. Kennedy CR *et al.* (1999) Salt-sensitivity hypertension and reduced fertility in mice lacking the prostaglandin EP₂ receptor. *Nat. Med.* 5:217-220.
 2. Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.
 3. Yang N *et al.* (2003) Cancer-associated immunodeficiency and dendritic cell abnormalities mediated by the prostaglandin EP₂ receptor. *J. Clin. Invest.* 111: 727–735.

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