eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ EP_3 Prostanoid Membrane Preparation

CATALOG NUMBER: HTS092M **QUANTITY:** 200 units

LOT NUMBER: SC240681 **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 prostaglandin PGE_2 causes pain, vasodilation, immunosuppression of T cells, bone resorption, and promotion of carcinogenesis. Four related GPCRs, EP_1, EP_2, EP_3 and EP_4, each bind to PGE_2, but the different G protein-coupling status of each receptor leads to distinct biological effects. Further diversity is generated by alternative splicing; the human gene for EP_3 generates 9 alternatively spliced mRNAs encoding 8 isoforms of EP_3 (Kotani *et al.*, 1997). These isoforms of EP_3 vary in sequence at their C-termini and differ in their ability to couple to G_s, G_q or G_i (Kotani *et al.*, 1995). EP_3 is required for fever induced by pyrogens, a response long attributed to prostaglandins by the antipyretic action of aspirin and other COX inhibitors (Ushikubi *et al.*, 1998). The EP_3 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 EP_3 interactions and its ligands. The membrane preparations exhibit a Kd of 1.9 nM for [^{3}H]-Prostaglandin E_2. With 1.5 nM [^{3}H]-PGE_2, 10 µg/well of EP_3 Membrane Prep yields greater than a 15-fold signal-to-background ratio.

APPLICATIONS:

Radioligand Binding Assay

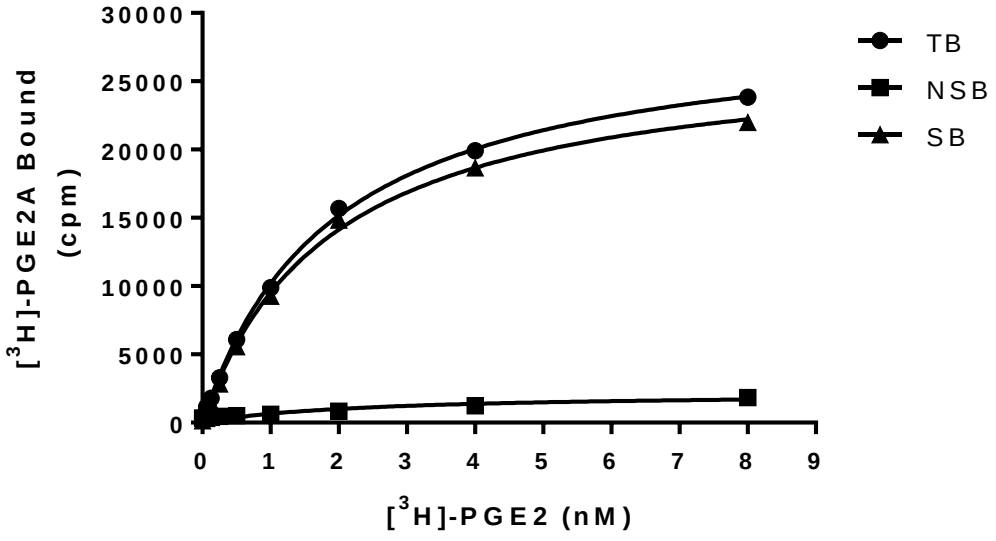

Figure 1. Saturation Binding for EP_3. 10 µg/well EP_3 Membrane Preparation was incubated with increasing amounts of [^{3}H]-Prostaglandin E_2 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled prostaglandin E_2. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample of lot SC240681.

Eurofins Pharma Bioanalytics Services US Inc.

15 Research Park Drive
St Charles MO 63304
USA

T | +1 844 522 7787
F | +1 636 362 7131
www.eurofins.com

Template version: 7. Modified Date: 27APR15. Modified by: JJP

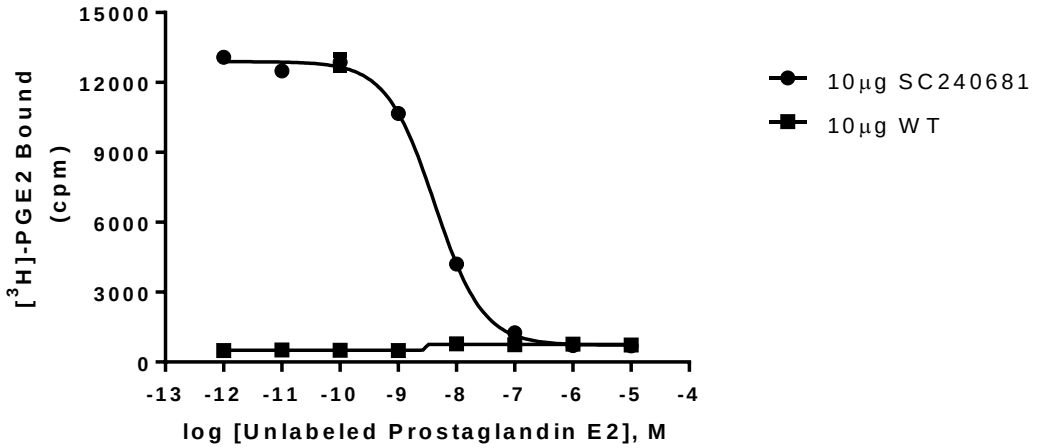

Figure 2. Competition Binding for EP_3. 10 µg/well of EP_3 or Wild Type (WT; Chem-1; Catalog # HTS000MC1) Membrane Preparation were incubated with 1.5 nM [^{3}H]-Prostaglandin E_2 and increasing concentrations of unlabeled prostaglandin E_2, and subjected to filtration binding. The data are from a representative sample of lot SC240681.

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{3}H]-PGE2 Binding: 14.6 pmol/mg protein
K_d for [^{3}H]-PGE2 Binding: 1.9 nM
Signal:Background: >15-fold

TRANSFECTION: Full-length human PTGER3 cDNA encoding splice variant 6 of EP_3 (Accession Number: NM_198716).

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15 and without endogenous EP_3 expression.

RECOMMENDED ASSAY CONDITIONS: Membranes were mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a non-binding 96-well plate, and incubated for 2 h. Prior to filtration, an FC 96-well harvest plate was coated with 0.33% polyethyleneimine for 30 min, and washed with 50 mM Tris, pH 7.4. The binding reactions were transferred to the filter plate and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells were then dried and counted for determination of receptor-associated radioligand binding.

Binding Buffer: 50 mM Tris, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, filtered and stored at 4°C

Radioligand: [^{3}H]-Prostaglandin E_2 (PerkinElmer # NET428)

Wash Buffer: 50 mM Tris, pH 7.4

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than a 15-fold signal:background ratio with [^{3}H]-Prostaglandin E_2 at 1.5 nM.

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 2 mg/mL in packaging buffer, dispensed at 1 mL per vial, 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. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Kotani M *et al.* (1995). Molecular cloning and expression of multiple isoforms of human prostaglandin E receptor EP3 subtype generated by alternative messenger RNA splicing: multiple second messenger systems and tissue-specific distributions. *Mol Pharmacol.* 48:869-879.

2. Kotani M *et al.* (1997). Structural organization of the human prostaglandin EP_3 receptor subtype gene (PTGER3). *Genomics* 40:425-434.

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

4. Ushikubi F *et al.* (1998). Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP_3. *Nature* 395:281-284.

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

Eurofins Pharma Bioanalytics Services US Inc. is an independent member of Eurofins Discovery Services.