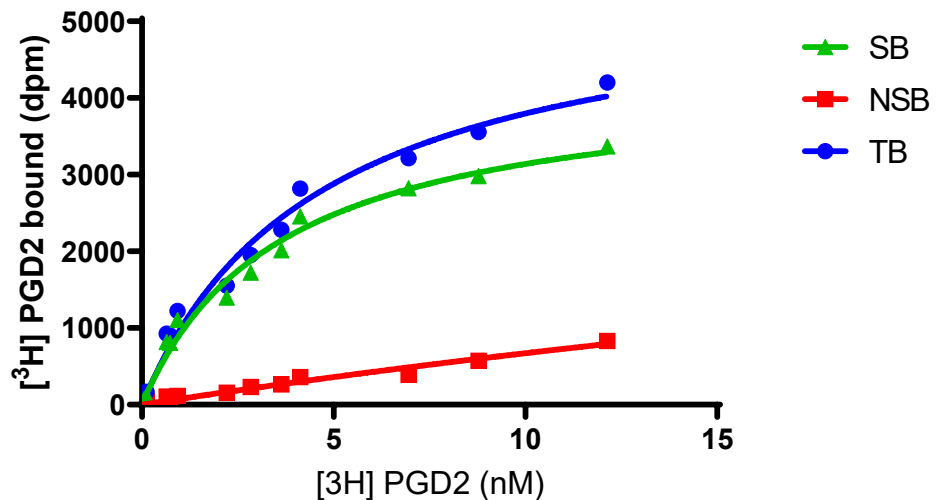


**PRODUCT DATASHEET**
**ChemiScreen™ DP Prostanoid Membrane Preparation**

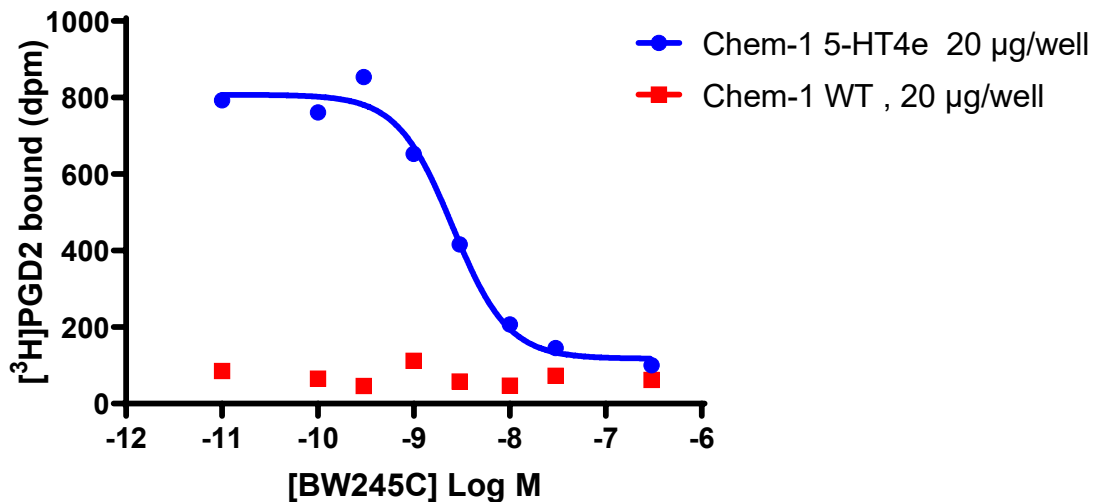
<b>CATALOG NUMBER:</b>	HTS091M	<b>QUANTITY:</b>	200 units
<b>LOT NUMBER:</b>	22M0713	<b>VOLUME/CONCENTRATION:</b>	1 mL, 2 mg/mL

**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 PGD<sub>2</sub> is produced by mast cells upon activation by allergens, and is present at high levels in allergic diseases. PGD<sub>2</sub> binds to two receptors, DP and CRTH2. DP activates G<sub>s</sub> to increase cAMP levels, and lack of DP results in reduced allergic response in animal models of bronchial asthma (Matsuoka *et al.*, 2000). DP 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 DP interactions and its ligands.

**APPLICATIONS:** Radioligand binding assay



**Figure 1. Saturation binding for DP.** 20 µg/well DP Membrane Preparation was incubated with increasing amount of <sup>3</sup>H-labeled PGD<sub>2</sub> in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled PGD<sub>2</sub>. Specific binding (SB) was determined by subtracting NSB from TB. Representative sample data.



**Figure 2. Competition binding for DP.** 20 µg/well DP Membrane Preparation and wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 8 nM <sup>3</sup>H-labeled PGD<sub>2</sub> and increasing concentrations of unlabeled BW245C. An 8 fold signal:background was obtained with 20 µg/well DP Membrane and  $\geq 3$ -fold signal:background can be obtained with 10 µg/well DP Membrane. Representative sample data.

**SPECIFICATIONS:** 1 unit = 10 µg  
 $B_{max}$  for [<sup>3</sup>H] PGD<sub>2</sub> binding: 0.544 pmol/mg protein  
 $K_d$  for [<sup>3</sup>H] PGD<sub>2</sub> binding: 3.6 nM  
Signal:background:  $\geq 3$ -fold

**TRANSFECTION:** Full-length human PTGDR cDNA encoding DP (Accession Number: NM\_000953)

**Species:** Human

**HOST CELLS:** Chem-1, an adherent mammalian cell line with minimum amount of endogenous DP 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, 0.5% BSA. 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 MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.2% BSA, filtered and stored at 4°C.

**Radioligand:** [<sup>3</sup>H] PGD<sub>2</sub> (Perkin Elmer#: NET616 )

**Wash Buffer:** 50 mM HEPES, pH 7.4, 500mM 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  $\geq 3$ -fold signal:background with <sup>3</sup>H-labeled PGD<sub>2</sub> at 8 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 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. Matsuoka T. *et al.* (2000) Prostaglandin D<sub>2</sub> as a mediator of allergic asthma. *Science* 287: 2013-2017.
2. Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.

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