

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN DP PROSTANOID RECEPTOR

CATALOG NUMBER:	HTS091M	QUANTITY:	200 units
LOT NUMBER:		VOLUME/CONCENTRATION PER VIAL:	2 mL, 1 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 PGD₂ is produced by mast cells upon activation by allergens, and is present at high levels in allergic diseases. PGD₂ binds to two receptors, DP and CRTH2. DP activates G_s to increase cAMP levels, and lack of DP results in reduced allergic response in animal models of bronchial asthma (Matsuoka *et al.*, 2000). Millipore's 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. The membrane preparations exhibit a K_d of 10.4 nM for [³H]-PGD₂. With 8 nM [³H]-PGD₂, 10 μg/well DP Membrane Prep typically yields greater than 4-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay and GTPγS binding.

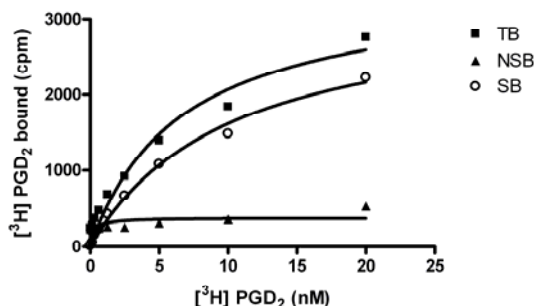


Figure 1. Saturation binding for DP. 5 μg/well DP Membrane Preparation was incubated with increasing amount of ³H-labeled PGD₂ in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled PGD₂. Specific binding (SB) was determined by subtracting NSB from TB.

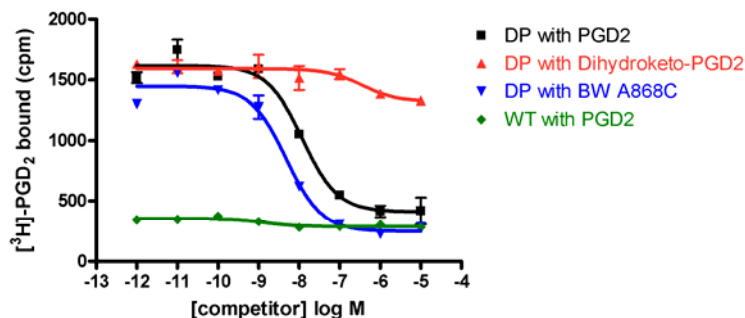


Figure 2. Competition binding for DP. 10 μg/well DP Membrane Preparation and wild-type Chem-1 Membrane Preparation (Millipore catalog # HTS000MC1) were incubated in a 96-well plate with 8 nM ³H-labeled

PGD₂ and increasing concentrations of unlabeled PGD₂, 13,14-dihydro-15-keto prostaglandin D₂, and BW A868C. More than 4- fold signal:background was obtained with BW A868C.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with DP membrane prep and unlabeled BW A868C.

	10 µg/well
Signal:background	5.8
Specific binding (cpm)	1197

Table 2. IC50 values for ligands obtained in a competition binding assay with DP membrane preparation

	IC50 (nM)
Prostaglandin D ₂	12.1
13,14-dihydro-15-keto prostaglandin D ₂	>10000
BW A868C	4.8

SPECIFICATIONS: 1 unit = 10 µg

B_{max} for [³H] PGD₂ binding: 5.3 pmol/mg protein

K_d for [³H] PGD₂ binding: ~10.39nM

TRANSFECTION: Full-length human PTGDR cDNA encoding DP (Accession Number: NM_000953)

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 (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 MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

Radioligand: [³H] PGD₂ (Perkin Elmer#: NET-616)

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 4-fold signal:background with ³H-labeled PGD₂ at 8 nM

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives. Packaging method: Membranes protein were adjusted to 0.5 mg/mL in 1 mL packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING:

Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

REFERENCES:

Matsuoka T. *et al.* (2000) Prostaglandin D₂ as a mediator of allergic asthma. *Science* 287: 2013-2017.
Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.

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