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PRODUCT DATASHEET

ChemiScreen™ CRTH2 Prostanoid Membrane Preparation

CATALOG NUMBER:	HTS031M	**QUANTITY:**	200 units
LOT NUMBER:	JH1818787	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND:

Chemoattractant receptor homologous molecule expressed on Th2 cells (CRTH2) is a GPCR related to FPR-like chemoattractant receptors that is highly expressed on Th2 cells, eosinophils, basophils, and type 2 cytotoxic T cells (Nagata *et al*., 1999). The most potent ligand for CRTH2 is prostaglandin D_2 (PGD_2), a major mast cell-derived allergic mediator (Hirai *et al*., 2001). Another GPCR, DP, is activated by PGD_2, and the relative contributions of DP and CRTH2 to PGD_2-mediated allergic disease are being elucidated. A small molecule antagonist of CRTH2, ramatroban, attenuates PGD_2-mediated bronchial hyperresponsiveness and antigen-induced inflammation (Sugimoto *et al*., 2003; Shichijo *et al*., 2003). CRTH2 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 PGD_2/CRTH2 interactions. The membrane preparations exhibit a Kd of 10 nM for [^{3}H]-Prostaglandin D_2. With 10 μg/well CRTH2 Membrane Prep and 10 nM [^{3}H]-Prostaglandin D_2, a greater than 8-fold signal-to-background ratio is obtained.

APPLICATIONS:

Radioligand binding assay, and GTPγS binding

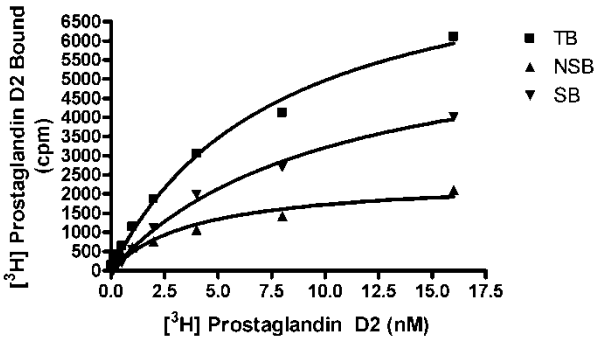

Figure 1. Saturation binding for CRTH2. 10 μg/well CRTH2 Membrane Preparation was incubated with increasing amount of ^{3}H-labeled Prostaglandin D_2 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled Prostaglandin D_2. Specific binding (SB) was determined by subtracting NSB from TB.

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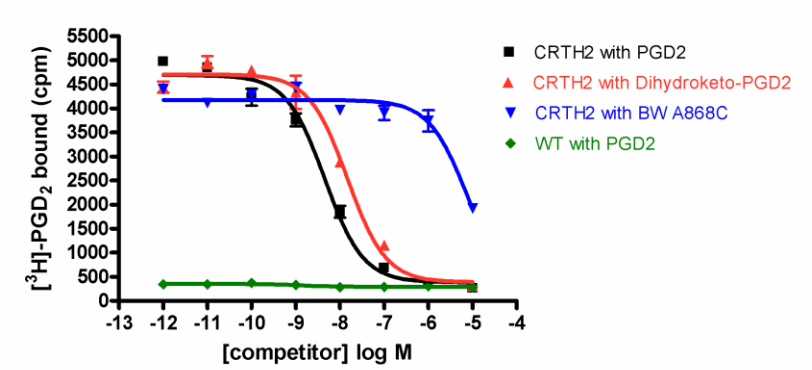

Figure 2. Competition binding for CRTH2. 10 µg/well CRTH2 Membrane Preparation (HTS031M) and Wild-Type Chem-1 Membrane Preparation (WT; cat. # HTS000MC1) were incubated with 10 nM ^{3}H-labeled Prostaglandin D_2 and increasing concentrations of unlabeled Prostaglandin D_2, 13,14-dihydro-15-keto prostaglandin D_2, and BW A868C. More than 8- fold signal:background was obtained with Prostaglandin D_2.

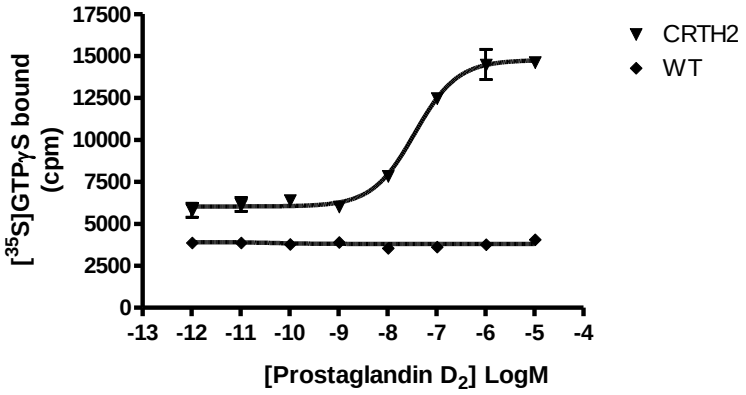

Figure 3. Binding of [^{35}S]-GTPγS to CRTH2 membrane preparation. 5 µg/well (0.5 units) CRTH2 Membrane Preparation (catalog # HTS020M2) and Wild-type Chem-1 Membrane Preparation (HTS000MC1) were incubated with 0.3 nM [^{35}S]-GTPγS, 0.5 µM GDP and increasing amounts of unlabeled Prostaglandin D_2. Bound radioactivity was determined by filtration and scintillation counting.

Table 1. Signal:background and specific binding values obtained in a competition radioligand binding assay with CRTH2 membrane preparation with unlabeled Prostaglandin D_2.

	10 µg/well
Signal:background	12.2
Specific binding	4307

Table 2. IC50 values for ligands obtained in a competition binding assay with CRTH2 membrane preparation.

	IC50 (nM)
Prostaglandin D_2	4.5
13,14-dihydro-15-keto prostaglandin D_2	14.7
BW A868C	7429

SPECIFICATIONS: 1 unit = 10 μg
B_{max}: 13.9 pmol/mg
K_d: 10 nM

Species: Human CRTH2 (Accession number AB008535)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous CRTH2 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: 50mM HEPES pH 7.0, 10mM $MnCl_2$, 1mM EDTA, 0.2% BSA, filtered and stored at 4°C.

Radioligand: [^{3}H] Prostaglandin D_2 (Amersham TRK734, PerkinElmer NET616)

Wash Buffer: 50mM HEPES pH 7.4, 0.5 M NaCl, filtered and stored at 4°C.

GTPγS ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [^{35}S]-GTPγS (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM $MgCl_2$/0.5 μM GDP in a nonbinding 96-well plate. Unlabeled ligand was added to the final concentration indicated in Figure 1 (final volume 100 μL), and incubated for 30 min at 30°C. The binding reaction is transferred to an FB filter plate (EMD Millipore MAHF B1H) previously prewetted with water. The plate is washed 3 times (1 mL per well per wash) with cold 10 mM sodium phosphate, pH 7.4, then dried and counted.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 8-fold signal:background with ^{3}H-labeled Prostaglandin D_2 at 10 nM in a radioligand binding assay.

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 inl 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. Hirai H *et al.* (2001) Prostaglandin D_2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J. Exp. Med.* 193: 255-261.

2. Nagata K *et al.* (1999) Selective expression of a novel surface molecule by human Th2 cells in vivo. *J. Immunol.* 162: 1278-1286.

3. Shichijo M *et al.* (2003) Chemoattractant receptor-homologous molecule expressed on Th2 cells activation in vivo increases blood leukocyte counts and its blockade abrogates 13:14-dihydro-15-keto-prostaglandin D_2-induced eosinophilia in rats. *J. Pharmacol. Exp. Ther*. 307: 518-525.

4. Sugimoto H *et al.* (2003) An orally bioavailable small molecule antagonist of CRTH2, ramatroban (BAY u3405), inhibits prostaglandin D_2-induced eosinophil migration in vitro. *J. Pharmacol. Exp. Ther.* 305: 347-352.