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CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN $CysLT_1$ CYSTEINYL LEUKOTRIENE RECEPTOR

CATALOG NUMBER:	HTS061M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	2 mL, 1 mg/mL

BACKGROUND:

The cysteinyl leukotrienes, leukotriene C_4, leukotriene D_4 and leukotriene E_4, are arachidonic acid derivatives modified by glutathione, Cys-Gly or Cys. Activated mast cells release cysteinyl leukotrienes, which cause smooth muscle contraction, airway constriction, and vascular permeability. The biological effects of the cysteinyl leukotrienes are mediated by two GPCRs, $CysLT_1$ and $CysLT_2$. A $CysLT_1$ selective antagonist, montelukast, is used clinically in the treatment of asthma (Brink *et al.*, 2003; Evans, 2002). Chemicon's $CysLT_1$ 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 $CysLT_1$ interactions and its ligands. The membrane preparations exhibit a Kd of 0.97nM for [^{3}H]-Leukotriene D_4 (LTD4). With 10.0ug/well $CysLT_1$ membrane prep and 1.5 nM [^{3}H]-Leukotriene D_4, a greater than 3-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay, and GTPγS binding.

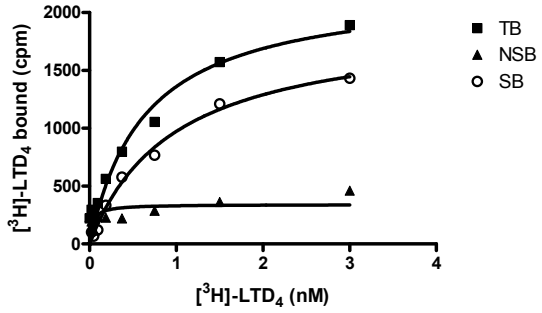

Figure 1. Saturation binding for $CysLT_1$ Receptor. 10.0 μg/well $CysLT_1$ Membrane Preparation was incubated with increasing amount of [^{3}H]-LTD_4 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled Cinalukast. Specific binding (SB) was determined by subtracting NSB from TB.

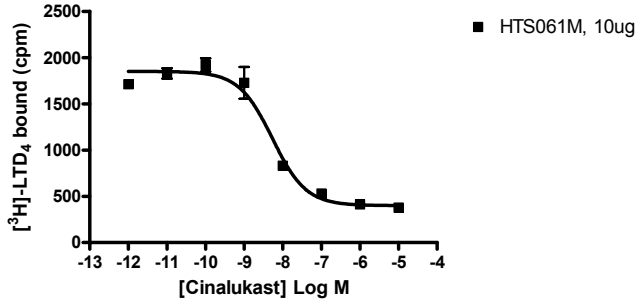

Figure 2. Competition binding for $CysLT_1$ Receptor. $CysLT_1$ Receptor Membrane Preparation (10μg/well) was incubated with 1.5 nM [^{3}H]-LTD_4 and increasing concentrations of unlabeled Cinalukast, and more than 3-fold signal:background was obtained.

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Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of $CysLT_1$ Receptor membrane prep.

	10 μg/well
Signal:background	4.62
Specific binding (cpm)	1451.4

SPECIFICATIONS: 1 unit = 10 μg membrane preparation
Bmax: 1.53 pmol/mg
K_d: 0.97 nM

Species: Full-length human CYSLTR1 cDNA (Accession Number: NM_006639)
HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous $CysLT_1$ Receptor 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 Tris, pH 7.4. 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 Tris, pH7.4, 20mM $CaCl_2$, 25mM $MgCl_2$, 10mM Glycine, 10mM L-Cysteine, filtered and stored at 4°C

Radioligand: [^{3}H] LTD_4 (Perkin Elmer#**:** NET-101)

Wash Buffer: 50 mM Tris, pH 7.4, filtered and stored at 4°C.

One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 3-fold signal:background with ^{3}H-labeled LTD4 at 1.5 nM.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris, pH 7.4, 10% glycerol with no preservatives. Packaging method: Membranes protein was adjusted to the indicated concentration in 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:

Brink C *et al.* (2003) International Union of Pharmacology XXXVII. Nomenclature for Leukotriene and Lipoxin Receptors. *Pharmacol. Rev.* 55: 195-227.

Evans JF (2002) Cysteinyl leukotriene receptors. *Prostaglandins Other Lipid Mediat.* 68-69:587-97.

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