

**CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN BLT₁ LEUKOTRIENE RECEPTOR**

CATALOG NUMBER:	HTS042M	QUANTITY:	200 units
LOT NUMBER:		VOLUME/CONCENTRATION PER VIAL:	1 mL, 2 mg/mL

BACKGROUND: BLT₁ receptor is a G_i-coupled GPCR that binds primarily to the eicosanoid leukotriene B₄. Macrophages, leukocytes and eosinophils express BLT₁, and in vivo, BLT₁ appears to mediate leukocyte migration and inflammation (reviewed in Brink *et al.*, 2003). Chemicon's BLT₁ 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 BLT₁ interactions with Leukotriene B₄. The membrane preparations exhibit a K_d of 1.3 nM for [³H]-Leukotriene B₄. With 10.0 µg/well BLT₁ Membrane Prep and 1 nM [³H]-Leukotriene B₄, greater than 4-fold signal:background is obtained.

APPLICATIONS: Radioligand binding assay, and GTPγS binding.

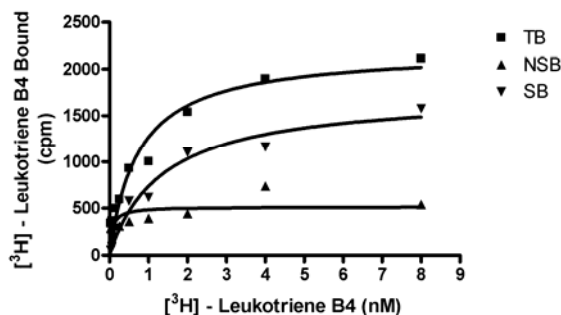


Figure 1. Saturation binding for BLT₁. 5 µg/well BLT₁ Membrane Preparation was incubated with increasing amount of ³H-labeled Leukotriene B₄ in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled Leukotriene B₄. Specific binding (SB) was determined by subtracting NSB from TB.

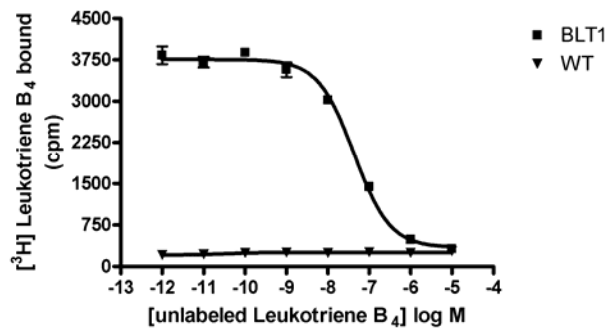


Figure 2. Competition binding for BLT $_1$. 10 $\mu\text{g}/\text{well}$ BLT $_1$ Membrane Preparation (HTS042M) and Wild-Type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated with 1 nM ^3H -labeled Leukotriene B_4 and increasing concentrations of unlabeled Leukotriene B_4 , and greater than 4-fold signal:background was obtained.

Table 1. Signal:background and specific binding in a competition binding assay with BLT₁ membrane preparation

	10 µg
Signal:background	10.9
Specific binding	3415

SPECIFICATIONS: 1 unit = 10 µg

B_{max}: 2.53 pmol/mg;K_d: 1.3 nMSpecies: Human BLT₁ (Accession number D89078)HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous BLT₁ 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, a GF/C 96-well filter plate 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.

PRESENTATION:Binding buffer: 50 mM Hepes, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°CRadioligand: [³H] Leukotriene (Perkin Elmer# NET852:)

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 ³H-labeled Leukotriene B₄ at 1 nM

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives.

Packaging method: Membranes protein were adjusted to the indicated concentration in 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:Brink C *et al.* (2003) International Union of Pharmacology XXXVII. Nomenclature for leukotriene and lipoxin receptors. *Pharmacol. Rev.* 55: 195-227.

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