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PRODUCT DATASHEET

ChemiScreen™ BLT_1 Bombesin Membrane Preparation

CATALOG NUMBER:	HTS042M	**QUANTITY:**	200 units
LOT NUMBER:	010714	**VOLUME/CONCENTRATION:**	1 mL, 2 mg/mL

BACKGROUND: BLT_1 receptor is a G_i-coupled GPCR that binds primarily to the eicosanoid leukotriene B_4. Macrophages, leukocytes and eosinophils express BLT_1, and in vivo, BLT_1 appears to mediate leukocyte migration and inflammation (reviewed in Brink *et al.*, 2003). EMD BLT_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 BLT_1 interactions with Leukotriene B_4.

APPLICATIONS: Radioligand binding assay

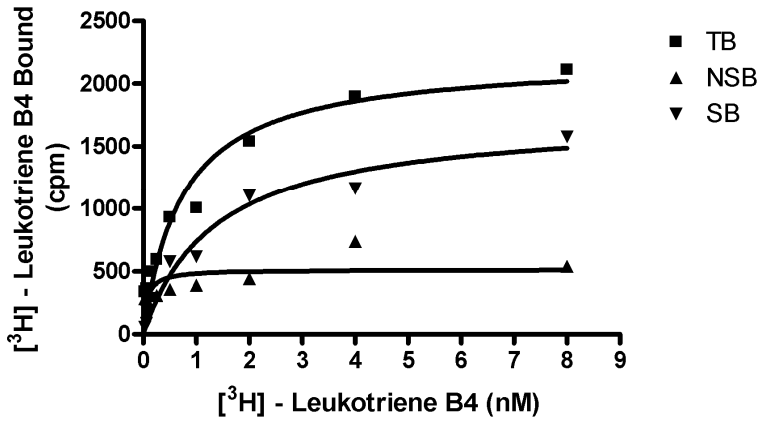

Figure 1. Saturation binding for BLT_1. 5 µg/well BLT_1 Membrane Preparation was incubated with increasing amount of ^{3}H-labeled Leukotriene B_4 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled Leukotriene B_4. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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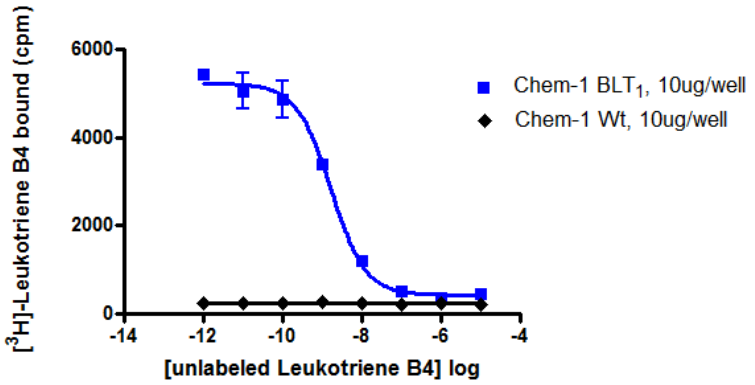

Figure 2. Competition binding for BLT_1. 10 mg/well BLT_1 Membrane Preparation (HTS042M) and Wild-Type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated with 1 nM ^{3}H-labeled Leukotriene B_4 and increasing concentrations of unlabeled Leukotriene B_4, and greater than 4-fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 10 μg membrane preparation
B_{max} 2.53 pmol/mg
K_d 1.3 nM
Signal:background: ≥4-fold

Species: Full-length human BLT_1 (Accession number D89078)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous BLT_1 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.

Binding buffer: 50 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C

Radioligand: [^{3}H] 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 ^{3}H-labeled Leukotriene B_4 at 1 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. Brink C *et al.* (2003) International Union of Pharmacology XXXVII. Nomenclature for leukotriene and lipoxin receptors. *Pharmacol. Rev.* 55: 195-227.