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PRODUCT DATASHEET

ChemiScreen™ BB1 Bombesin Membrane Preparation

CATALOG NUMBER: HTS123M **QUANTITY:** 200 units

LOT NUMBER: **VOLUME/CONCENTRATION:** 1 mL, 1 mg/mL

BACKGROUND:

Bombesin, a bioactive peptide first identified in amphibian skin, is related to two mammalian peptides, *gastrin*-releasing peptide (GRP) and neuromedin B (NMB). A family of 3 GPCRs, including NMB-R (BB_1), GRP-R (BB_2) and BRS-3 (BB_3), mediate the biological effects of the peptides. The receptors differ in their affinities for the peptides; BB_2 binds to GRP with 50-300-fold greater affinity than to NMB, whereas BB_1 binds to NMB with 10-800-fold greater affinity than to GRP (Tokita *et al*., 2004). Binding of ligand to BB_1 activates G_q to increase intracellular calcium concentrations. The CNS is a major site of NMB and BB_1 expression, and BB_1 appears to be involved in thermoregulation (Ohki-Hamazaki *et al*., 2005). BB1 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 BB1 interactions with neuromedin B. The membrane preparations exhibit a Kd of 0.35-0.43 nM for [^{125}I]-Bombesin. With 5 µg/well BB1 Membrane Prep and 0.3 nM [^{125}I]-bombesin, a greater than 40-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay, and GTPγS binding

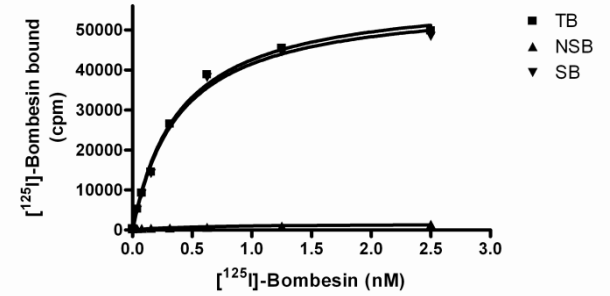

Figure 1. Saturation binding for BB1. 5 µg/well BB1 Membrane Preparation was incubated with increasing amount of [^{125}I]-bombesin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 5000-fold excess unlabeled neuromedin B. Specific binding (SB) was determined by subtracting NSB from TB.

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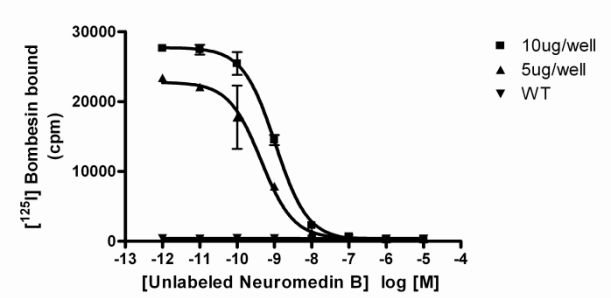

Figure 2. Competition binding for BB1. BB1 Membrane Preparation (5 or 10 µg /well) or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.3 nM [^{125}I]-bombesin and increasing concentrations of unlabeled neuromedin B, and more than 40- fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of BB1 membrane prep.

	10 µg/well	5 µg/well
Signal:background	82.5	74.8
Specific binding (cpm)	27377	22443

SPECIFICATIONS: 1 unit = 5 µg membrane preparation
B_{max} 3.1 pmol/mg
K_d 0.39 nM

Species: Full-length human NMBR cDNA encoding BB_1 (Accession Number: NM_002511)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous BB1 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: [^{125}I] bombesin (Perkin Elmer # NEX258)

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 40-fold signal:background with ^{125}I-labeled bombesin at 0.3 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^{o}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. Ohki-Hamazaki H *et al.* (2005) Development and function of bombesin-like peptides and their receptors. *Int. J. Dev. Biol.* 49: 293-300.

2. Tokita K *et al.* (2004) Molecular basis of the selectivity of gastrin-releasing peptide receptor for gastrin-releasing peptide. *Mol. Pharmacol.* 61: 1435-1443.

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