

PRODUCT DATASHEET

ChemiScreen™ BB3 Bombesin Membrane Preparation

CATALOG NUMBER: HTS160M **QUANTITY:** 200 units
LOT NUMBER: 2037899 **VOLUME/CONCENTRATION:** 2 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 (BB₁), GRP-R (BB₂) and BRS-3 (BB₃), mediate the biological effects of the peptides. The receptors differ in their affinities for the peptides; BB₂ binds to GRP with 50-300-fold greater affinity than to NMB, whereas BB₁ binds to NMB with 10-800-fold greater affinity than to GRP (Tokita *et al.*, 2004). Binding of ligand to BB₁ activates G_q to increase intracellular calcium concentrations. The CNS is a major site of NMB and BB₁ expression, and BB₁ 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.

APPLICATIONS: Radioligand binding assay

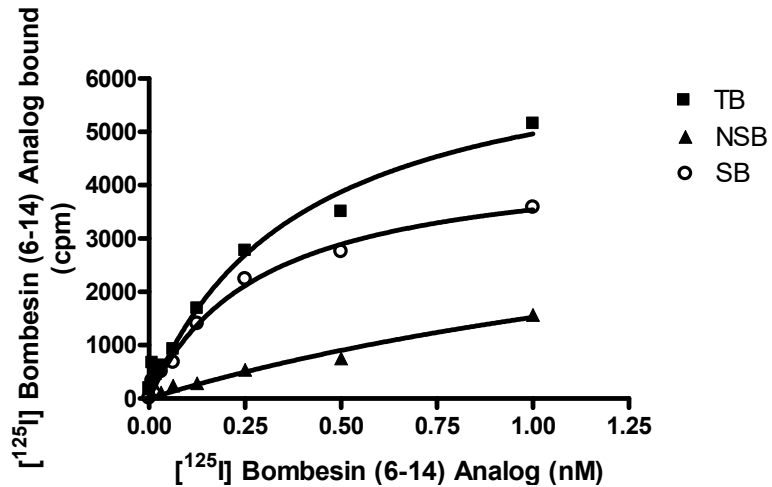


Figure 1. Saturation binding for BB₃. 5 µg/well BB₃ Membrane Preparation was incubated with increasing amount of ¹²⁵I-labeled Bombesin (6-14) Analog in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Bombesin (6-14) Analog. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

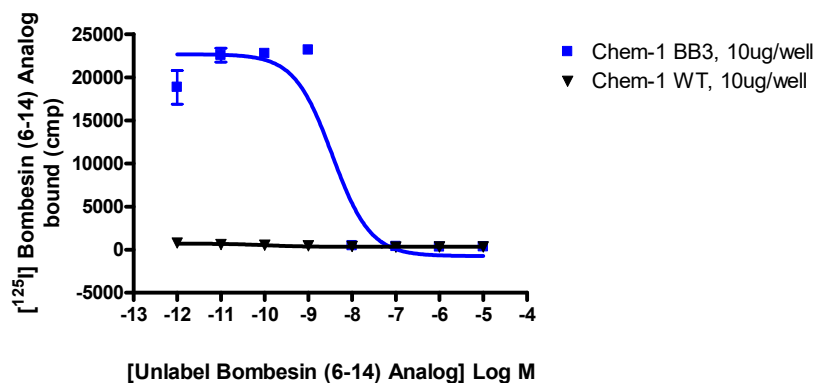


Figure 2. Competition binding for BB3. BB₃ Membrane Preparation and WT-Chem-1 Membrane Preparation (Millipore HTS000MC1), each at 10 µg/well in a 96-well plate, was incubated with 0.3 nM ¹²⁵I-labeled Bombesin (6-14) Analog and increasing concentrations of unlabeled Bombesin (6-14) Analog. The reaction was then subjected to filtration binding, and greater than 5-fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 10 µg membrane preparation
 B_{max} 0.95 pmol/mg
 K_d 0.3 nM

Species: Full length human BRS3 encoding BB3 (Accession number NM_001727)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous BB3 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 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 MgCl₂, 1 mM CaCl₂, 0.2% BSA, 0.2 mg/ml bacitracin, 20 µg/ml leupeptin, 20 µg/ml chymostatin, 1 Protease Inhibitor cocktail Tablets (Roche Cat. No. 11 873 580 001) for each 50 ml binding buffer.

Radioligand: [¹²⁵I]-Bombesin (6-14) Analog (Perkin Elmer NEX377)

Wash Buffer: 50 mM Hepes, pH 7.4, 500 mM NaCl, 0.1% BSA, 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 5-fold signal:background with ¹²⁵I-labeled Bombesin (6-14) Analog 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

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. Matsumoto K and Iijima H (2003) Sibutramine sensitivity assay revealed a unique phenotype of bombesin BB3 receptor-deficient mice. *Eur. J. Pharmacol.* 473: 41-46.
2. Ohki-Hamazaki H et al. (2005) Development and function of bombesin-like peptides and their receptors. *Int. J. Dev. Biol.* 49: 293-300.

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