

PRODUCT DATASHEET
ChemiScreen™ BB2 Bombesin Membrane Preparation

CATALOG NUMBER:	HTS084M	QUANTITY:	200 units
LOT NUMBER:	21F0301	VOLUME/CONCENTRATION:	1 mL, 0.5 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. 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. Binding of ligand to BB₂ activates Gq to increase intracellular calcium concentrations. GRP stimulates release of gastrin from endocrine cells and stimulates smooth muscle activity in the gastrointestinal tract. In addition, binding of GRP to BB₂ stimulates proliferation of a variety of cell types, and has been implicated in the progression of small cell lung cancer and other malignancies. The CNS is also a major site of GRP expression, and GRP and BB₂ are involved in the circadian clock, conditioned fear, and food intake (Ohki-Hamazaki et al., 2005). Chemicon's BB₂ 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 BB₂ interactions and its ligands. The membrane preparations exhibit a K_d of 0.114 nM for [¹²⁵I]-Tyr4-Bombesin. With 0.3nM [¹²⁵I]-Tyr4-Bombesin, 5 µg/well or 10 µg/well BB₂ Membrane Prep typically yield greater than 20-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

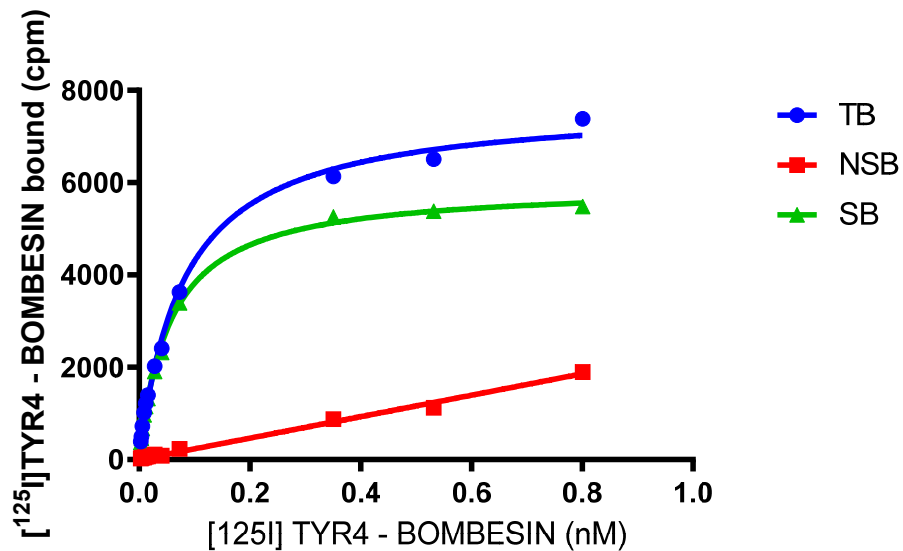


Figure 1. Saturation binding for BB₂. 1.5 µg/well BB₂ Membrane Preparation was incubated with increasing amount of [¹²⁵I]-bombesin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of unlabeled Bombesin at 1 µM. Specific binding (SB) was determined by subtracting NSB from TB.

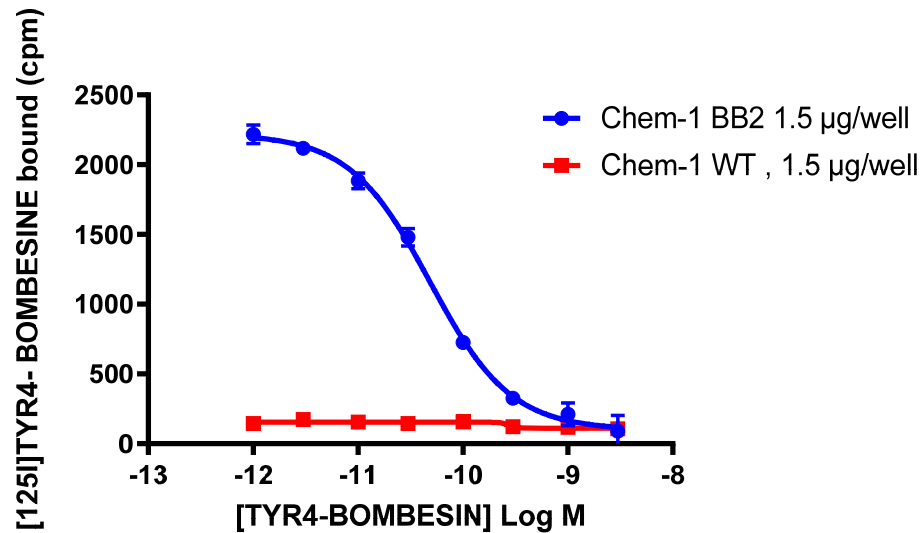


Figure 2. Competition binding for BB₂. 0.5 µg /well BB₂ Membrane Preparation or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.1 nM [¹²⁵I]-bombesin and increasing concentrations of unlabeled bombesin, and more than 40- fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 1.5 µg membrane preparation
 B_{max} 0.23 pmol/mg
 K_d 0.056 nM

Species: Full-length human GRPR cDNA encoding BB₂ (Accession Number: M73481)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous BB₂ 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 1h. Prior to filtration, a GF/B 96-well filter plate is coated with 50 mM Tris-HCl, pH 7.4 + 0.3% polyethyleneimine. Binding reaction is transferred to the filter plate, and washed 4 times (~1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 20 mM Hepes/NaOH (pH 7.4), 3 mM MgCl₂, 1 mM EDTA and 0.3% BSA

Radioligand: [¹²⁵I] bombesin (Perkin Elmer # NEX258)

Wash Buffer: 50 mM Tris-HCl pH 7.4

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 ¹²⁵I-labeled bombesin.

- 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. 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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