

**CHEMISCREEN[™] MEMBRANE PREPARATION
RECOMBINANT HUMAN BB₂ BOMBESIN RECEPTOR**

CATALOG NUMBER: HTS084M **QUANTITY:** 200 units
LOT NUMBER: **VOLUME/CONCENTRATION PER VIAL:** 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 G_q 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 and GTPγS binding.

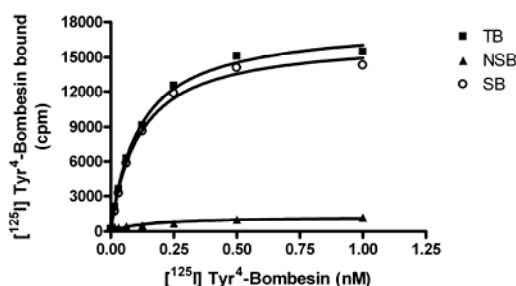


Figure 1. Saturation binding for BB₂. 5 µg/well BB₂ Membrane Preparation was incubated with increasing amount of ¹²⁵I-labeled Tyr⁴-Bombesin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled ICI 216,140. Specific binding (SB) was determined by subtracting NSB from TB.

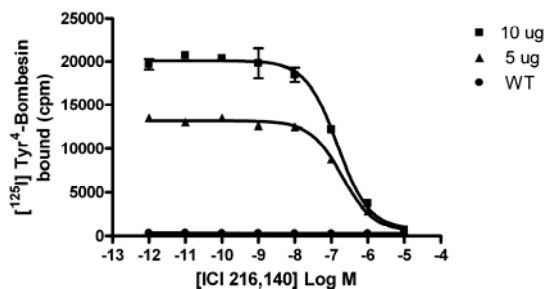


Figure 2. Competition binding for BB₂. BB₂ Membrane Preparation (5 and 10 µg/well in a 96-well plate)

were incubated with 1.5nM ¹²⁵I-labeled Tyr⁴-Bombesin and increasing concentrations of unlabeled ICI 216,140, and subjected to filtration binding.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of BB₂ membrane prep.

	10 µg/well	5 µg/well
Signal:background	24	23
Specific binding (cpm)	19,260	12,610

SPECIFICATION: Bmax: 1.28 pmol/mg; Kd: 0.114 nM

TRANSFECTION: 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 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 Tris, pH 7.4, 10 mM MgCl₂, 1 mM EDTA, filtered and stored at 4°C

Radioligand: [¹²⁵I]-Tyr⁴-Bombesin (Perkin Elmer#:NEX-258)

Wash Buffer: 50 mM Tris, pH 7.4 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 10-fold signal:background with ¹²⁵I labeled Bombesin at 0.3 nM

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives. Packaging method: Membranes protein were adjusted to 0.5 mg/ml in 1 ml packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING:

Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

REFERENCES:

Ohki-Hamazaki H *et al.* (2005) Development and function of bombesin-like peptides and their receptors. *Int. J. Dev. Biol.* 49: 293-300.

For research use only; not for use as a diagnostic.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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