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CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT BB_3 BOMBESIN RECEPTOR

CATALOG NUMBER:	HTS160M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	2 mL, 1.0 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 GRP-R (BB_1), NMB-R (BB_2) and BRS-3 (BB_3), mediate the biological effects of the peptides (Ohki-Hamazaki *et al*., 2005). BB_3 differs from the others by its low affinity for bombesin. Although an endogenous ligand for BB_3 has yet to be identified, a synthetic nonselective bombesin-like peptide [H-D-Phe^6, β-Ala^{11}, Phe^{13}, Nle^{14}]-bombesin-(6-14)-nonapeptide amide (Bombesin (6-14) Analog) activates BB_3 with high potency. BB_3-null mice have an obese phenotype (Matsumoto and Iijima, 2003). Millipore's BB_3 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 BB_3 receptor interactions with its ligand. The membrane preparations exhibit a Kd of 0.23nM for [^{125}I]-Bombesin (6-14) Analog. With 10 μg/well BB_3 Membrane Prep and 0.3 nM [^{125}I]-Bombesin (6-14) Analog, a greater than 3-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay, and GTPγS binding.

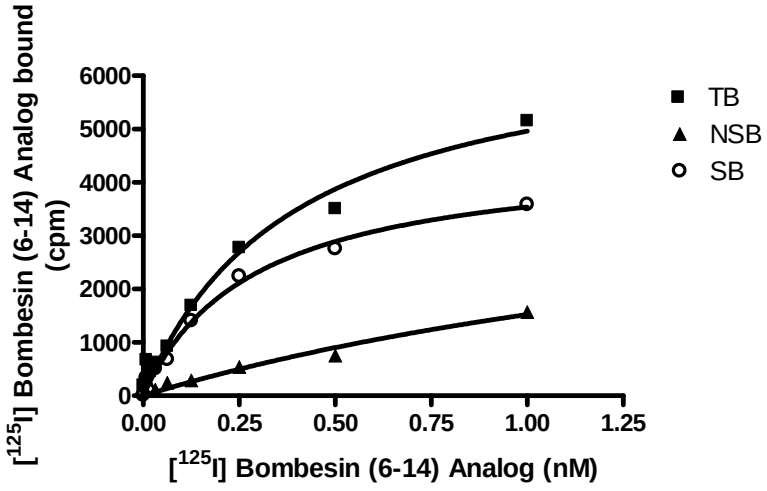

Figure 1. Saturation binding for BB_3. 5 μg/well BB_3 Membrane Preparation was incubated with increasing amount of ^{125}I-labeled Bombesin (6-14) Analog in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled Bombesin (6-14) Analog. Specific binding (SB) was determined by subtracting NSB from TB.

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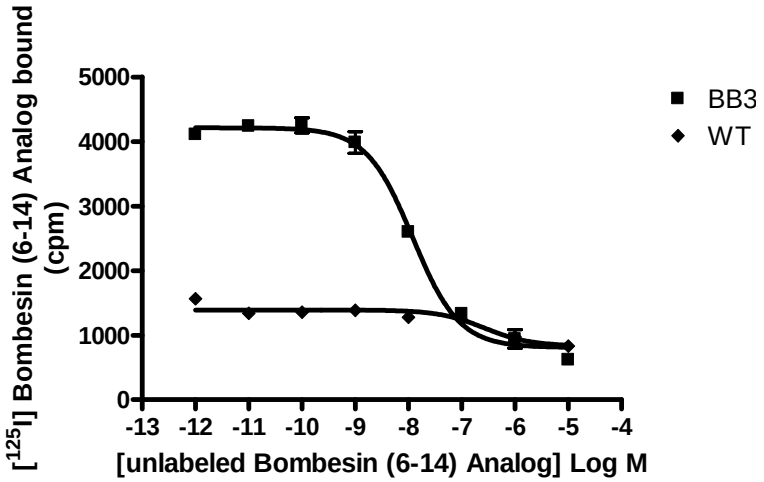

Figure 2. Competition binding for BB_3. BB_3 Membrane Preparation and WT-Chem-1 Membrane Preparation (Millipore HTS000MC1), each at 10 μg/well in a 96-well plate, was incubated with 0.3 nM ^{125}I-labeled Bombesin (6-14) Analog and increasing concentrations of unlabeled Bombesin (6-14) Analog. The reaction was then subjected to filtration binding, and greater than 3-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with BB_3 Receptor membrane prep.

	10 μg/well
Signal:background	5.2
Specific binding (cpm)	3403

SPECIFICATIONS: 1 unit = 10 μg membrane preparation
Bmax: 0.95 pmol/mg
K_d: 0.3 nM

Species: Full length human BRS3 encoding BB_3 (Accession number NM_001727)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous BB_3 Receptor 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 $MgCl_2$, 1 mM $CaCl_2$, 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: [^{125}I]-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.

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One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 3-fold signal:background with ^{125}I-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: Membranes protein was adjusted to the indicated concentration in 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:

Matsumoto K and Iijima H (2003) Sibutramine sensitivity assay revealed a unique phenotype of bombesin BB_3 receptor-deficient mice. *Eur. J. Pharmacol.* 473: 41-46.

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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