

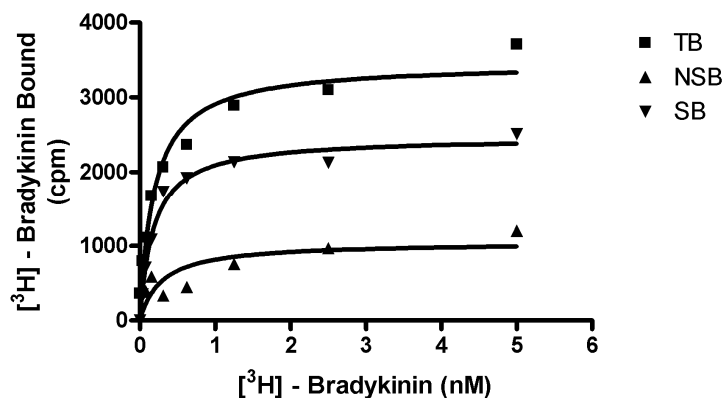
## CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN B<sub>2</sub> BRADYKININ RECEPTOR

**CATALOG NUMBER:** HTS041M      **QUANTITY:** 200 units

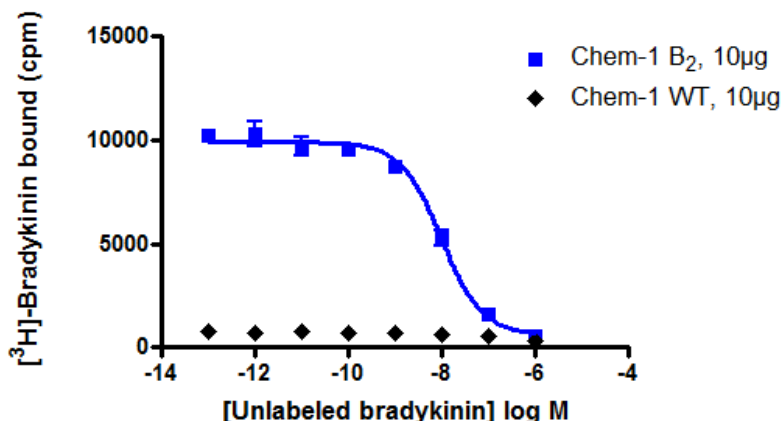
**LOT NUMBER:**      **VOLUME/CONCENTRATION PER VIAL:** 1 mL, 2 mg/mL

**BACKGROUND:** Bradykinins bind and activate two GPCRs, termed B<sub>1</sub> and B<sub>2</sub> (gene names BDKRB1 and BDKRB2, respectively), which signal through G<sub>q</sub> and G<sub>i</sub>. B<sub>2</sub> is widely expressed in a constitutive fashion, whereas B<sub>1</sub> expression is induced during inflammation. B<sub>2</sub> appears to mediate much of the activity of kinins in vasodilation, arthritis, edema, and nociception (Leeb-Lundberg *et al.*, 2005). Chemicon's B<sub>2</sub> 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 B<sub>2</sub> interactions with bradykinin. The membrane preparations exhibit a K<sub>d</sub> of 0.18 nM for [<sup>3</sup>H]-bradykinin. With 10 µg/well, B<sub>2</sub> Membrane Prep and 0.4 nM [<sup>3</sup>H]-bradykinin, a greater than 10-fold signal-to-background ratio is obtained.

**APPLICATIONS:** Radioligand binding assay.



**Figure 1. Saturation binding for B<sub>2</sub>.** 2.5 µg/well B<sub>2</sub> Membrane Preparation was incubated with increasing amount of <sup>3</sup>H-labeled bradykinin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled bradykinin. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.



**Figure 2. Competition binding for B<sub>2</sub>.** 10 µg/well B<sub>2</sub> Membrane Preparation (Cat. # HTS041M) and Wild-Type Chem-1 Membrane Preparation (Cat. # HTS000MC1) were incubated with 10 nM <sup>3</sup>H-labeled Bradykinin and increasing concentrations of unlabeled bradykinin, and more than 10- fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 10 µg  
 B<sub>max</sub>: 2.73 pmol/mg;  
 K<sub>d</sub>: 0.18 nM  
 Signal:background: >10-fold

Species: Human BDKRB2 cDNA encoding B<sub>2</sub> (Accession number M88714)  
 HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous B<sub>2</sub> 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<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.2% BSA, filtered and stored at 4°C

Radioligand: [<sup>3</sup>H] Bradykinin, [2,3-prolyl-3,4-<sup>3</sup>H(N)] (Perkin Elmer #NET-706)

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 a unit is the amount of membrane that will yield greater than 10-fold signal:background with <sup>3</sup>H-labeled bradykinin at 0.4 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:** Leeb-Lundberg L.M.F. *et al.* (2005) International Union of Pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences. *Pharmacol. Rev.* 57: 27-77.

**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.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

©2006 - 2012: EMD Millipore Corporation. All rights reserved. No part of these works may be reproduced in any form without permission in writing.