

PRODUCT DATASHEET
ChemiScreen™ B2 Bradykinin Membrane Preparation

CATALOG NUMBER: HTS041M **QUANTITY:** 200 units
LOT NUMBER: 21K1806 **VOLUME/CONCENTRATION:** 1 mL, 2 mg/mL

BACKGROUND: Bradykinins bind and activate two GPCRs, termed B₁ and B₂ (gene names BDKRB1 and BDKRB2, respectively), which signal through G_q and G_i. B₂ is widely expressed in a constitutive fashion, whereas B₁ expression is induced during inflammation. B₂ appears to mediate much of the activity of kinins in vasodilation, arthritis, edema, and nociception (Leeb-Lundberg *et al.*, 2005). B₂ 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 B₂ interactions with bradykinin. The membrane preparations exhibit a K_d of 1.3 nM for [³H]-bradykinin.

APPLICATIONS: Radioligand binding assay

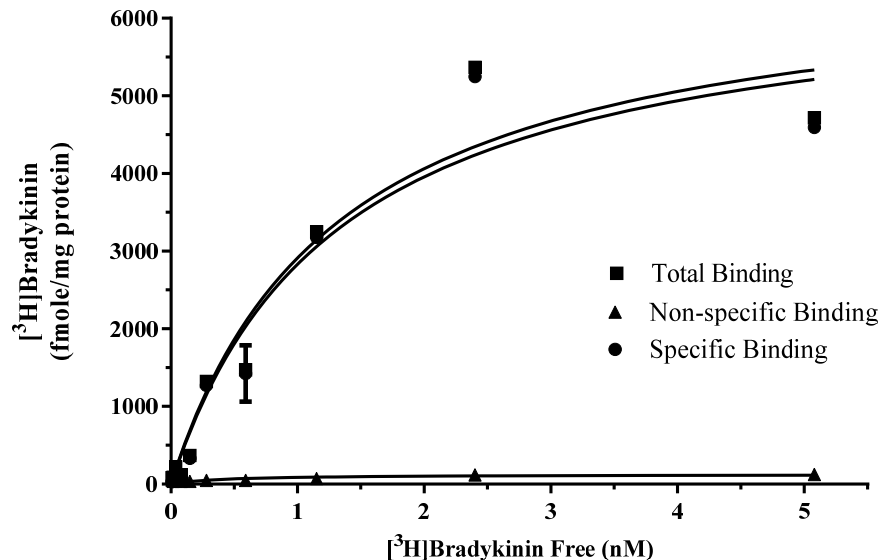


Figure 1. Saturation Binding for B₂. 10 μg/well of B₂ Membrane Preparation were incubated with increasing amounts of [³H]-Bradykinin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled bradykinin. Specific binding (SB) was determined by subtracting NSB from TB. The sample data are from a representative lot.

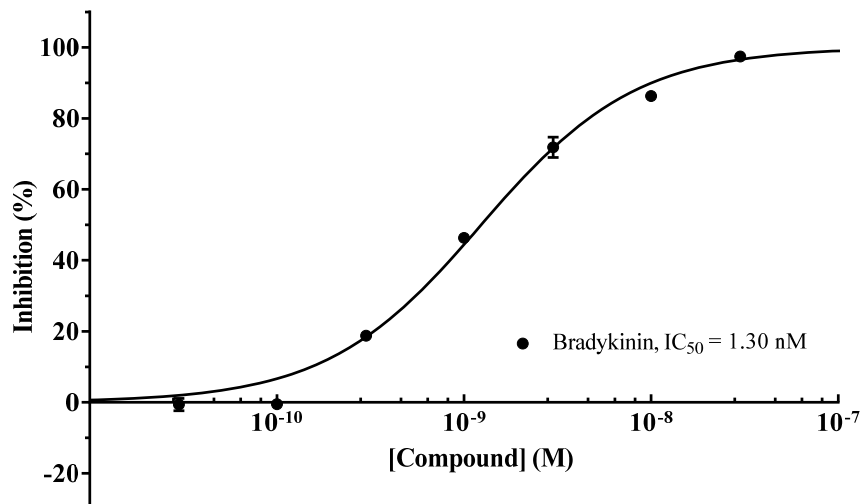


Figure 2. Competition Binding for B₂. 10 μg/well of B₂ Membrane Preparation or Wild-Type Chem-1 Membrane Preparation (Cat. # HTS000MC1) were incubated with 0.5 nM [³H]-Bradykinin and increasing concentrations of unlabeled bradykinin, and more than a 7-fold signal:background ratio was obtained. The sample data are from a representative lot.

SPECIFICATIONS: 1 unit = 10 μg
 B_{max} for [³H]-Bradykinin Binding: 6.5 pmol/mg protein
 K_d for [³H]-Bradykinin Binding: 1.3 nM
 Signal:background: >7

Species: Human BDKRB2 cDNA encoding B₂ (Accession number M88714)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous B₂ 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 non-binding 96-well plate, and incubated for 1-2 h at room temperature. Prior to filtration, a GF/C 96-well filter plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. The binding reactions are transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells are then dried and counted for determination of receptor-associated radioligand binding.

Binding buffer: 50 mM HEPES, pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

Radioligand: [³H]-Bradykinin (PerkinElmer # NET706)

Wash Buffer: 50 mM HEPES, pH 7.4, 500 mM NaCl, 0.1% BSA, filtered and stored at 4°C.

- 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. 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..

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