

**CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN CCK₂ CHOLECYSTOKININ RECEPTOR**

CATALOG NUMBER: HTS083M **QUANTITY:** 200 units
LOT NUMBER: **VOLUME/CONCENTRATION :** 2mL, 0.5 mg/mL

BACKGROUND: Cholecystokinins are a series of peptides of heterogeneous length (5 to 58 amino acids) that are derived from preprocholecystokinin and are found in gastrointestinal tissues and the central nervous system. Gastrin is a related peptide with 5 C-terminal amino acids identical to those of cholecystokinin. Two GPCRs, CCK₁ (CCK_A) and CCK₂ (CCK_B), bind to CCK and/or gastrin to mediate the biological effects of the peptides. CCK₂ binds to CCK and gastrin with similar affinity, whereas CCK₁ selectively binds sulfated CCK. Binding of ligands to CCK₂ stimulates mobilization of intracellular calcium by activation of G_{q/11}. In the periphery, CCK₂ is present in the stomach, where it mediates gastrin-stimulated gastric acid secretion. In the CNS, CCK₂ has been implicated in anxiety, depression, schizophrenia, depression and opioid analgesia (Noble *et al.*, 1999). Chemicon's CCK₂ 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 CCK₂ interactions and its ligands. The membrane preparations exhibit a K_d of 0.47 nM for [¹²⁵I]-cholecystokinin octapeptide (CCK-8). With 0.3 nM [¹²⁵I]-CCK-8, 5 or 10 µg/well CCK₂ Membrane Prep yields greater than 20-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay and GTPγS binding.

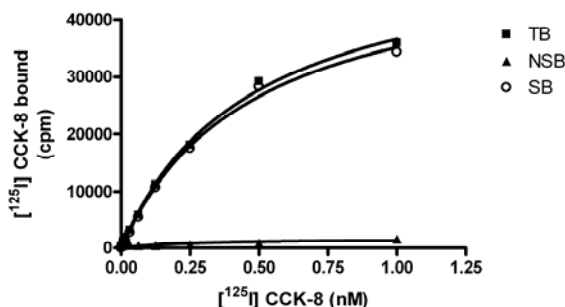


Figure 1. Saturation binding for CCK₂. 5 µg/well CCK₂ Membrane Preparation was incubated with increasing amount of ¹²⁵I labeled CCK-8 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled gastrin. Specific binding (SB) was determined by subtracting NSB from TB.

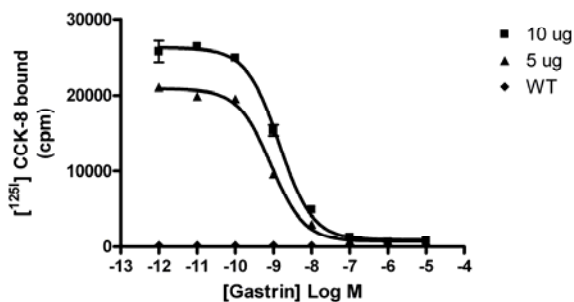


Figure 2. Competition binding for CCK₂. CCK₂ Membrane Preparation (5 and 10 µg/well in a 96-well plate) were incubated with 0.3 nM ¹²⁵I labeled CCK-8 and increasing concentrations of unlabeled gastrin, and subjected to filtration binding.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of CCK₂ membrane prep.

	10 µg/well	5 µg/well
Signal:background	27.4	27.8
Specific binding (cpm)	25302	20111

SPECIFICATIONS: 1 unit = 5 µg
 B_{max} for [¹²⁵I] CCK-8 binding: 3.47 pmol/mg protein;
 K_d for [¹²⁵I] CCK-8 binding: ~ 0.47 nM

TRANSFECTION: Full-length human CCKB cDNA encoding CCK₂ (Accession Number: NM_176875)

HOST CELLS: Chem-1, an adherent mammalian cell line with no endogenous CCK₂ 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 MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

Radioligand: [¹²⁵I]-CCK-8 (Perkin Elmer#:NEX-203)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM 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 20-fold signal:background with ¹²⁵I labeled CCK-8 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: Noble F *et al.* (1999) International Union of Pharmacology. XXI. Structure, distribution, and functions of cholecystokinin receptors. *Pharmacol. Rev.* 51: 745-781.

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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USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
 Australia +61 3 9839 2000 • Germany +49-6192-207300 • ISO Registered worldwide
 www.chemicon.com • custserv@chemicon.com • techserv@chemicon.com