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PRODUCT DATASHEET

ChemiScreen™ CCK_2 Cholecystokinin Membrane Preparation

CATALOG NUMBER:	HTS083M	**QUANTITY:**	200 units
LOT NUMBER:	SC539801	**VOLUME/CONCENTRATION:**	1 mL, 1 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, CCK_1 (CCK_A) and CCK_2 (CCK_B), bind to CCK and/or gastrin to mediate the biological effects of the peptides. CCK_2 binds to CCK and gastrin with similar affinity, whereas CCK_1 selectively binds sulfated CCK. Binding of ligands to CCK_2 stimulates mobilization of intracellular calcium by activation of $G_{q/11}$. In the periphery, CCK_2 is present in the stomach, where it mediates gastrin-stimulated gastric acid secretion. In the CNS, CCK_2 has been implicated in anxiety, depression, schizophrenia, depression and opioid analgesia (Noble *et al.*, 1999). CCK_2 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 CCK_2 interactions and its ligands. The membrane preparations exhibit a Kd of 0.47 nM for [^{125}I]-cholecystokinin octapeptide (CCK8). With 0.3 nM [^{125}I]-CCK8, 5 µg/well of CCK_2 Membrane Prep yields greater than a 20-fold signal-to-background ratio.

APPLICATIONS:

Radioligand Binding Assay

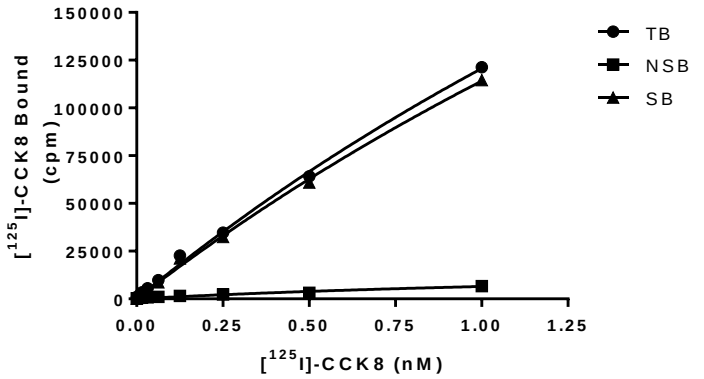

Figure 1. Saturation Binding for CCK_2. 5 µg/well CCK_2 Membrane Preparation was incubated with increasing amounts of [^{125}I]-CCK8 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled gastrin. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample from lot SC539801.

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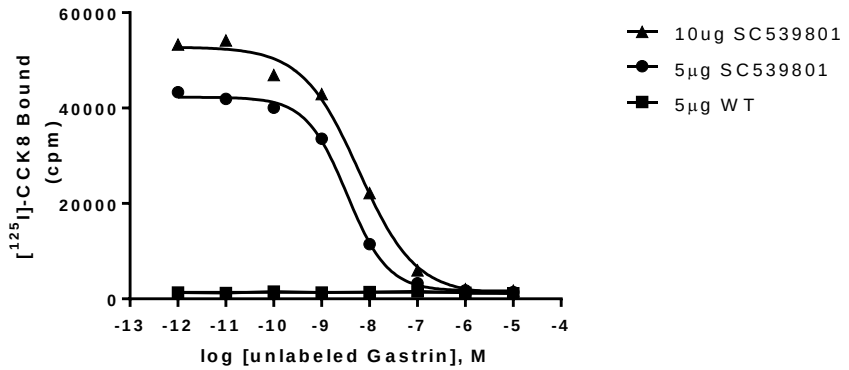

Figure 2. Competition Binding for CCK_2. CCK_2 Membrane Preparation (5 and 10 µg/well in a 96-well plate) were incubated with 0.3 nM [^{125}I]-CCK8 and increasing concentrations of unlabeled gastrin, and subjected to filtration binding. The data are from a representative sample from lot SC539801.

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{125}I]-CCK8 binding: 51.5 pmol/mg protein
K_d for [^{125}I]-CCK8 binding: 4.5 nM
Signal:background: >20-fold

TRANSFECTION: Full-length human CCKB cDNA encoding CCK_2 (Accession Number: NM_176875).

HOST CELLS: Chem-1, an adherent mammalian cell line with no endogenous CCK_2 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 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.

Binding Buffer: 50 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C.

Radioligand: [^{125}I]-CCK8 (PerkinElmer # NEX203).

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 a 20-fold signal:background ratio with [^{125}I]-CCK8 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: Membrane proteins were adjusted to 1.0 mg/mL 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. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Noble F *et al.* (1999). International Union of Pharmacology. XXI. Structure, distribution, and functions of cholecystokinin receptors. *Pharmacol. Rev.* 51:745-781.