eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ CCK_1 Cholecystokinin Membrane Preparation

CATALOG NUMBER:	HTS184M	**QUANTITY:**	200 units
LOT NUMBER:	2207299	**VOLUME/CONCENTRATION:**	1 mL, 2 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_1 selectively binds sulfated CCK, whereas CCK_2 binds to CCK and gastrin with similar affinity. Binding of ligands to CCK_1 stimulates mobilization of intracellular calcium by activation of $G_{q/11}$. CCK_1 receptors in the periphery are primarily localized in the pancreas, gallbladder, pylorus, intestine where they are responsible for the regulation of diverse digestive processes. They are also present in select areas of the peripheral nervous system (vagus nerve), and the CNS where they mediate the satiety effects of CCK, regulate an increase in dopamine release, and antagonize opioid analgesia (Noble *et al.*, 1999). CCK_1 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_1 interactions and its ligands. The membrane preparations exhibit a Kd of 0.5 nM for [^{125}I]-CCK-8 sulfated. With 10.0ug/well CCK_1 membrane prep and 0.5 nM [^{125}I]-CCK-8 sulfated, a greater than 4-fold signal-to-background ratio was obtained.

APPLICATIONS:

Radioligand binding assay

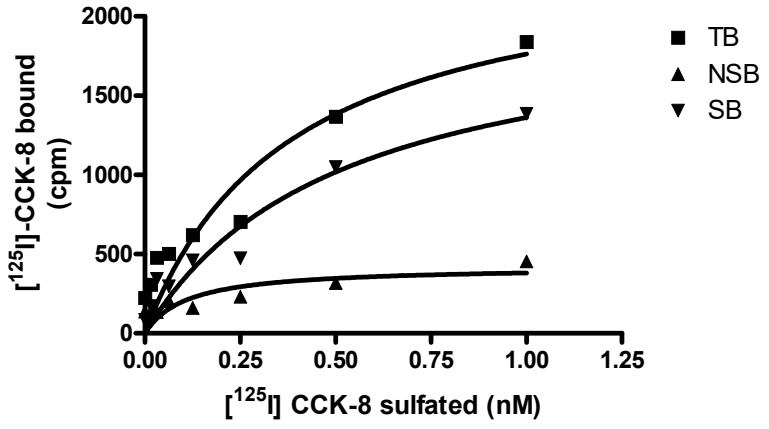

Figure 1. Saturation binding for CCK_1 Receptor. 5.0 µg/well CCK_1 Membrane Preparation was incubated with increasing amount of [^{125}I]-CCK-8 sulfated in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled A71623. Specific binding (SB) was determined by subtracting NSB from TB.

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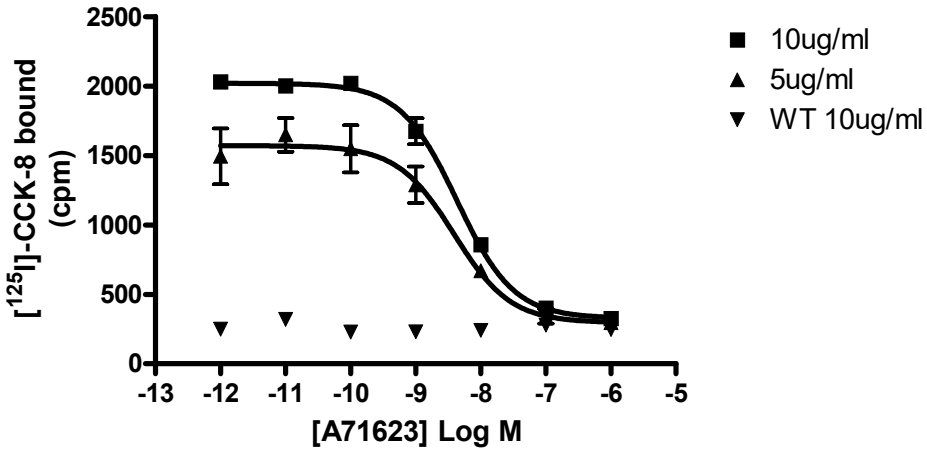

Figure 2. Competition binding for CCK_1 Receptor. CCK_1 Receptor Membrane Preparation (5.0 or 10µg/well) or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.5 nM [^{125}I]-CCK-8 sulfated and increasing concentrations of unlabeled A71623, and more than 4-fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{3}H]-Nociceptin binding: 0.15 pmol/mg
K_d for [^{3}H]-Nociceptin binding: 0.5 nM
Signal:background: 4-fold

TRANSFECTION: Full-length CCK_1 Receptor (Accession number NM_000730)

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

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 blocked 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.

Note: Due to the acidic property of the labeled sulfated CCK-8, filter plates coated with PEI bind the labeled ligand nonspecifically and result in elevated backgrounds.

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] –CCK-8 sulfated (Perkin Elmer # NEX203)

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 an unit is the amount of membrane that will yield greater than 4-fold signal:background with ^{125}I-labeled CCK-8 at 0.5 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 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. Noble F *et al.* (1999) International Union of Pharmacology. XXI. Structure, distribution, and functions of cholecystokinin receptors. *Pharmacol. Rev.* 51: 745-781.