

PRODUCT DATASHEET
ChemiScreen™ AMY₁ Calcitonin Membrane Preparation

CATALOG NUMBER:	HTS127M	QUANTITY:	200 units
LOT NUMBER:	SC20181030	VOLUME/CONCENTRATION:	1 mL, 1 mg/mL

BACKGROUND: The calcitonin family of peptides, which includes calcitonin, two calcitonin-related gene peptides (CGRPs), adrenomedullin, and amylin, share a disulfide-bonded loop, an amphipathic α -helix, and an amidated C-terminus. The peptides influence a variety of physiological effects, including vascular tone, food intake and bone metabolism. The biological effects of the peptides are mediated by two class B GPCRs, the CT and CL receptors (Poyner *et al.*, 2002). The pharmacology of the CT and CL receptors is strongly influenced by their association with a family of three receptor activity modifying proteins (RAMPs), which are 14-17 kD single pass transmembrane proteins. When expressed with no modifying proteins, the CT receptor binds with high affinity only to calcitonin. The CT receptor in complex with RAMP proteins binds amylin with high affinity, as well as calcitonin and α CGRP; the CT receptor complexes with RAMP1, RAMP2 and RAMP3 are termed AMY₁, AMY₂ and AMY₃. Amylin (also known as islet amyloid polypeptide) is produced by pancreatic beta cells. Experiments with amylin-deficient mice indicate that amylin and its receptors control food intake and pain perception. Pramlintide, a synthetic analog of amylin, is used clinically for glucose control in diabetics. AMY₁ 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 AMY₁ interactions with hCGRP. The membrane preparations exhibit a K_d of 0.18 nM for [¹²⁵I]-hCGRP. With 5 μ g/well of AMY₁ Membrane Prep and 0.2 nM [¹²⁵I]-hCGRP, a greater than 10-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand Binding Assay

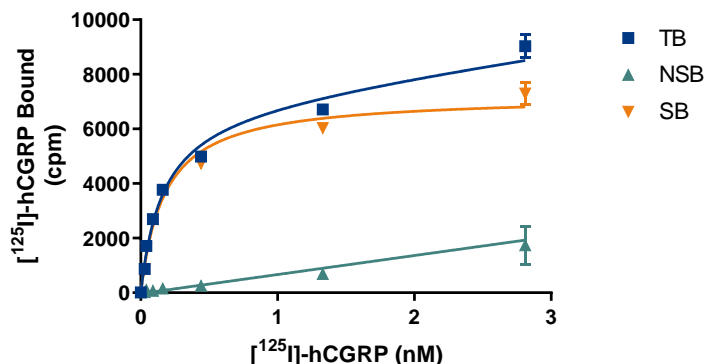


Figure 1. Saturation Binding for AMY₁. 5 μ g/well AMY₁ Membrane Preparation was incubated with increasing amount of [¹²⁵I]-hCGRP in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 500-fold excess unlabeled α CGRP. Specific binding (SB) was determined by subtracting NSB from TB.

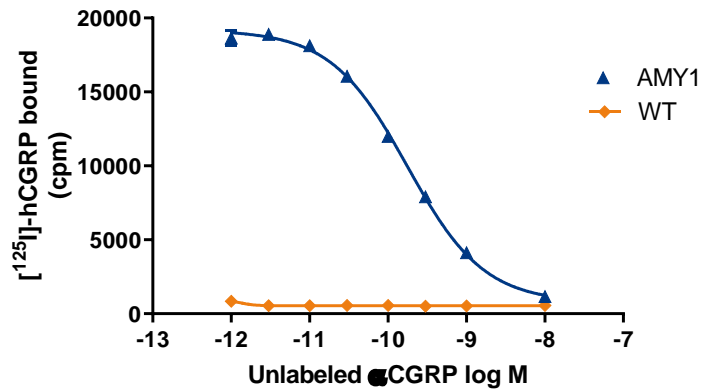


Figure 2. Competition Binding for AMY₁. AMY₁ Membrane Preparation (5 μg/well) or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.2 nM [¹²⁵I]-hCGRP and increasing concentrations of unlabeled αCGRP, and more than 10-fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 5 μg
 B_{max}: 0.30 pmol/mg
 K_d: 0.18 nM
 Signal:Background: >10-fold

Species: Full-length human CALCR (isoform 2) and RAMP1 cDNAs encoding AMY₁ (Accession Numbers: NM_001742 and NM_005855).

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous AMY₁ 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 50 mM 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] hCGRP (PerkinElmer # NEX354)

Wash Buffer: 50 mM HEPES, pH 7.4, 150 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 [¹²⁵I] hCGRP at 0.2 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. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Poyner DR *et al.* (2002). International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. *Pharmacol. Rev.* 54:233-246.

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