

PRODUCT DATASHEET
ChemiScreen™ CGRP1 Calcitonin Membrane Preparation

CATALOG NUMBER:	HTS172M	QUANTITY:	200 units
LOT NUMBER:	22J1404	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, CT receptor (calcitonin receptor) and CL receptor (calcitonin receptor-like receptor) (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. CGRP (calcitonin gene-related peptide) induces vasodilation, and its activity is primarily mediated by CGRP1, composed of CL in complex with RAMP1 (Hasbak *et al.*, 2003). As a consequence of its role as a vasodilator, CGRP acting via CGRP1 appears to play an important role in hypertension (Deng and Li, 2005). The CGRP1 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 CGRP1 interactions with CGRP.

APPLICATIONS:

Radioligand Binding Assay

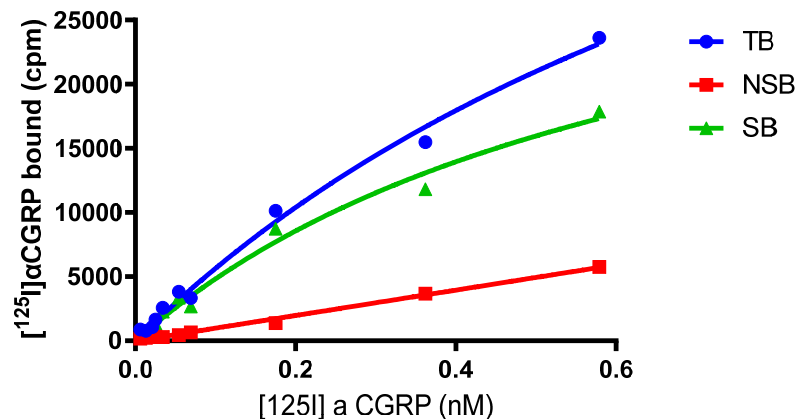


Figure 1. Saturation Binding for CGRP1. 5 μ g/well of CGRP1 Membrane Preparation were incubated with increasing amounts of [125 I]-hCGRP in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 2000-fold excess of unlabeled α CGRP. Specific binding (SB) was determined by subtracting NSB from TB. The sample data are from a representative lot.

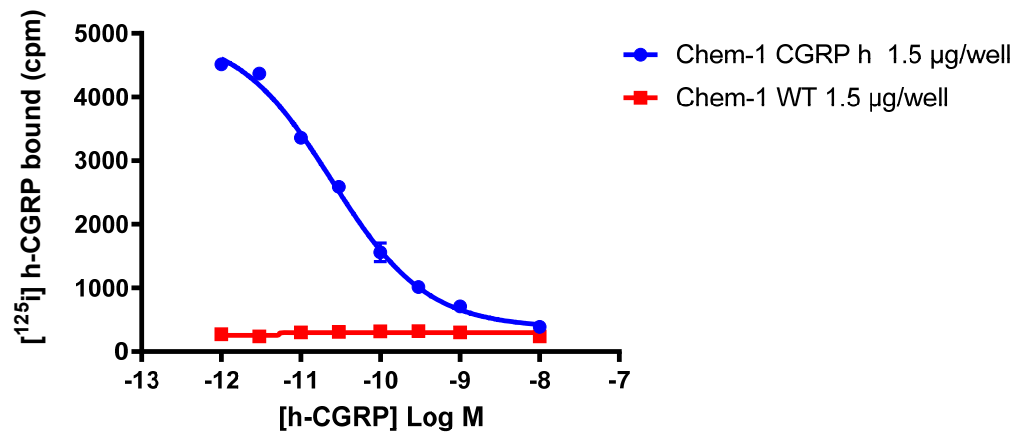


Figure 2. Competition Binding for CGRP1. CGRP1 Membrane Preparation (1.5 µg/well) or Wild-Type Chem-1 Membrane Preparation (WT; Catalog # HTS000MC1) were incubated with 1 nM [¹²⁵I]-hCGRP and increasing concentrations of unlabeled αCGRP, and more than a 5-fold signal:background ratio was obtained. The sample data are from a representative lot.

SPECIFICATIONS: 1 unit = 5 µg
 B_{max} for [¹²⁵I]-hCGRP Binding: 7.8 pmol/mg protein
 K_d for [¹²⁵I]-hCGRP Binding: 1.0 nM
Signal:Background: ≥5-fold

TRANSFECTION: Full-length human CALCRL and RAMP1 cDNAs encoding CGRP1 (Accession Numbers: NM_005795 and NM_005855).

HOST CELLS: Chem-1, an adherent mammalian cell line with no detectable endogenous CGRP1 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 at room temperature for 2 h. Prior to filtration, an FC 96-well harvest 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 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, 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 5-fold signal:background ratio with [¹²⁵I]-hCGRP at 1 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, dispensed at 1 mL per vial, 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. Deng PY and Li YJ (2005). Calcitonin gene-related peptide and hypertension. *Peptides* 26:1676-85.
 2. Hasbak P *et al.* (2003). Investigation of CGRP receptors and peptide pharmacology in human coronary arteries. Characterization with a nonpeptide antagonist. *J. Pharmacol. Exp. Ther.* 304(1):326-33.
 3. 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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