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PRODUCT DATASHEET

ChemiScreen™ CRF1 Neuropeptide Membrane Preparation

CATALOG NUMBER:	HTS023M	**QUANTITY:**	200 units
LOT NUMBER:	SC20190321	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND: The CRF1 receptor is a G_s-coupled GPCR expressed in the brain and pituitary gland that binds to several neuropeptides, including corticotropin-releasing factor (CRF) and urocortin, and the amphibian peptide sauvagine (Chen *et al*., 1993; Dautzenberg and Hauger, 2002; Bale and Vale, 2004). CRF plays a predominant role in stress response mediated by the hypothalamic-pituitary-adrenal axis, and alterations in CRF and its receptors CRF1 and CRF2 appear to be linked to depression and anxiety (Holsboer, 1999; Bale and Vale, 2004). A number of small molecule antagonists of the CRF1 receptor have been characterized, including R121919, SC241, NBI27914, antalarmin, DMP-696, and CP 154,526. When delivered in animal models of psychiatric disorders, these antagonists display effectiveness in reducing stress-related behaviors (Kehne and De Lombaert, 2002). CRF1 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 CRF1 interactions with its ligands. The membrane preparations exhibit a Kd of 1.76 nM for [^{125}I]-sauvagine.

APPLICATIONS: Radioligand binding assay

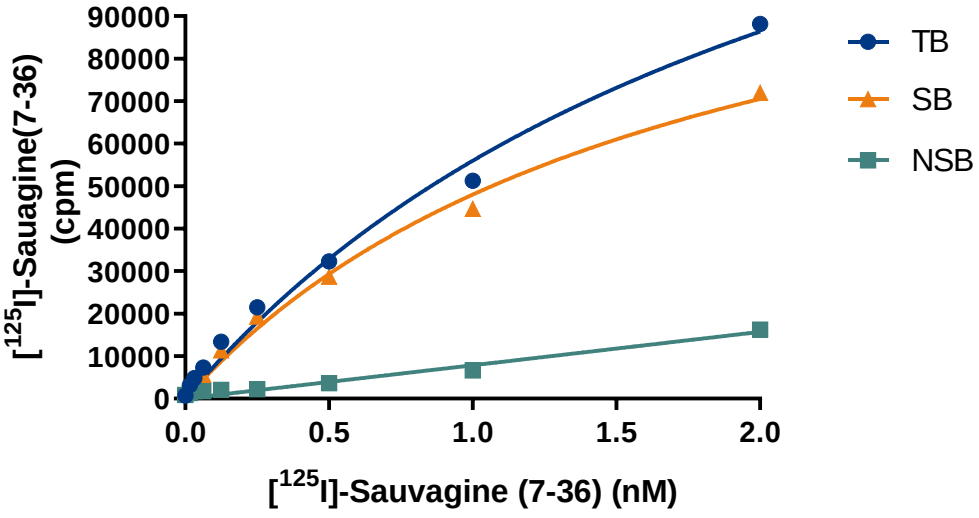

Figure 1. Saturation binding for CRF1. 5 µg/well CRF1 Membrane Preparation was incubated with increasing amount of ^{125}I-labeled Sauvagine in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled sauvagine. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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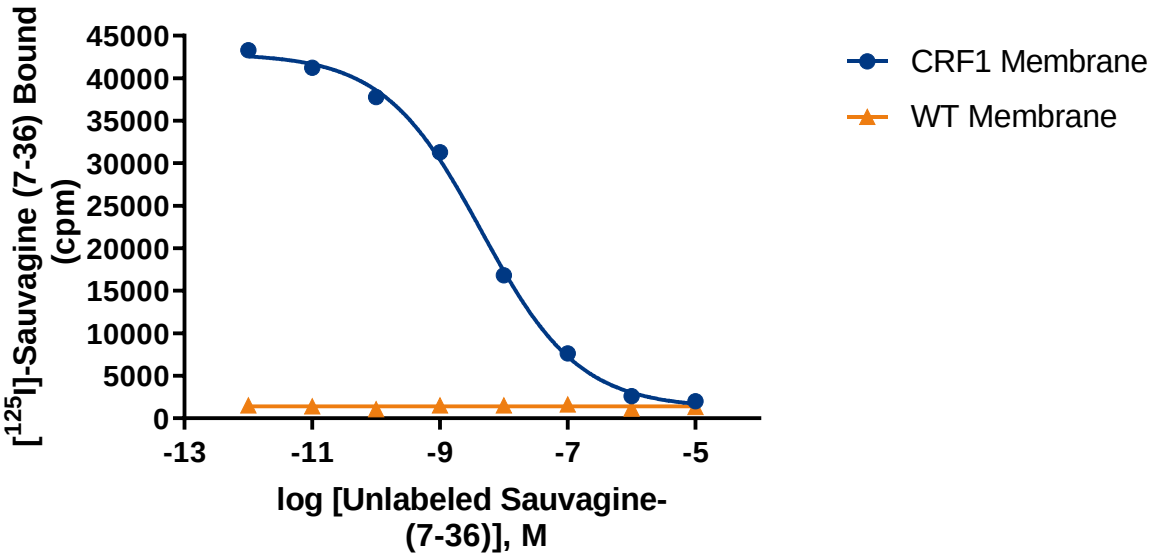

Figure 2. Competition binding for CRF1. 5 µg/well CRF1 Membrane Preparation (HTS023M) was incubated with 0.50 nM ^{125}I-labeled Sauvagine and increasing concentrations of unlabeled sauvagine, and more than 10- fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 5 µg
B_{max}: 10.88 pmol/mg
K_d: 1.76 nM
Signal:background: ≥10-fold

Species: Human CRF1 (Accession number X72304)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous CRF1 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 (Costar 3605), 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 $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C.

Radioligand: [^{125}I] sauvagine (Perkin Elmer#: NEX306)

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 10-fold signal:background with ^{125}I-labeled Sauvagine at 0.50 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 the indicated concentration in 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. Bale TL and Vale WW (2004) CRF and CRF receptors: role in stress responsivity and other behaviors. *Annu. Rev. Pharmacol. Toxicol.* 44: 525-557.
2. Chen R. *et al*. (1993) Expression cloning of a human corticotropin-releasing factor receptor. *Proc. Natl. Acad. Sci. USA* 90: 8967-8971.
3. Dautzenberg FM and Hauger RL (2002) The CRF peptide family and their receptors: yet more partners discovered. *Trends Pharmacol. Sci.* 23: 71-77.
4. Holsboer F (1999) The rationale for cotricotropin-releasing hormone receptor (CRH-R) antagonists to treate depression and anxiety. *J. Psychiatr. Res.* 33: 181-214.
5. Kehne J and De Lombaert S (2002) Non-peptidic CRF1 receptor antagonists for the treatment of anxiety, depression and stress disorders. *Curr. Drug Targets CNS Neurol. Disord.* 1: 467-493.

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