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PRODUCT DATASHEET

ChemiScreen™ CRF2 Neuropeptide Membrane Preparation

CATALOG NUMBER:	HTS024M	**QUANTITY:**	200 units
LOT NUMBER:	2326012	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

The corticotropin-releasing factor receptors, CRF1 and CRF2, are Gs-coupled GPCRs expressed in the brain, blood vessels and intestine that bind to several neuropeptides, including corticotropin-releasing factor (CRF) and urocortin, and the amphibian peptide sauvagine (Lovenberg et al., 1995; Bale and Vale, 2004). In addition, two peptides, urocortin II (Ucn II) and urocortin III (Ucn III), bind selectively and with high affinity to CRF2 (Lewis et al., 2001). The CRF peptides and their receptors play important roles in stress mediated by the hypothalamic-pituitary-adrenal axis in animal models, and possibly in depression and anxiety in humans, although the contributions of CRF1 and CRF2 appear to be distinct (Bale and Vale, 2004; Risbrough et al., 2004) In addition, CRF1 and CRF2 differentially alter feeding behavior, gastric motility and vascular tone (Zorrilla et al., 2004; Martinez et al., 2004; Wiley and Davenport, 2004). Millipore's CRF2 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 CRF2 interactions with its ligands. The membrane preparations exhibit a Kd of 0.65 nM for [^{125}I]-sauvagine. With 5 µg/well CRF2 Membrane Prep and 0.35 nM [^{125}I]-sauvagine, a greater than 20-fold signal-to-background ratio is obtained.

APPLICATIONS:

Radioligand binding assay

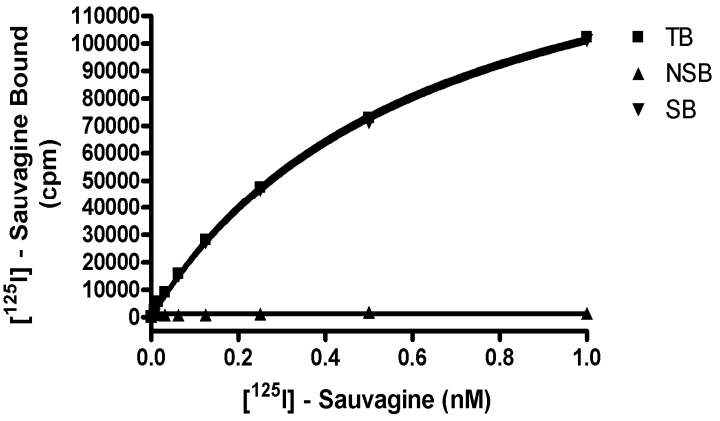

Figure 1. Saturation binding for CRF$_2$. 10 µg/well CRF$_2$ Membrane Preparation was incubated with increasing amount of ^{125}I-labeled Sauvagine in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled Urocortin II. Specific binding (SB) was determined by subtracting NSB from TB.

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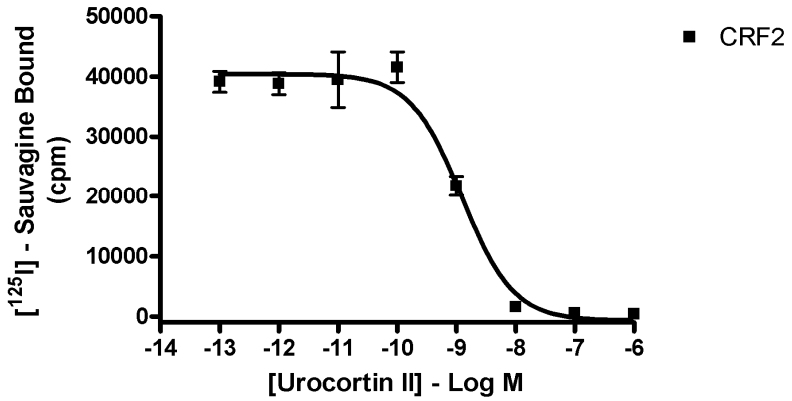

Figure 2. Competition binding for CRF_2. 5.0 µg/well CRF_2 Membrane Preparation (HTS024M) was incubated with 0.35 nM ^{125}I-labeled Sauvagine and increasing concentrations of unlabeled Urocortin II, and more than 20- fold signal:background was obtained.

SPECIFICATIONS: 1 unit = 5 µg
B_{max}: 9.04 pmol/mg
K_d: 0.65 nM
Signal:background: ≥20-fold

Species: Full-length human CRHR2 cDNA encoding CRF2 (Accession number NM_001883)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous CRF_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 1-2 h. Prior to filtration, an FC 96-well harvest plate (Millipore cat. # MAHF C1H) 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 20-fold signal:background with ^{125}I-labeled Sauvagine at 0.35 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. Lewis K et al. (2001) Identification of urocortin III, an additional membrer of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. Proc. Natl. Acad. Sci. USA 98: 7570-7575.
3. Lovenberg TW et al. (1995) Cloning and characterization of a functionally distinct corticoptropin-releasing factor receptor subtype from rat brain. Proc. Natl. Acad. Sci. USA 92: 836-840.
4. Martinez V et al. (2004) Central CRF, urocortins and stress increase colonic transit via CRF1 receptors while activation of CRF2 receptors delays gastric transit in mice. J. Physiol. 556: 221-234.
5. Risbrough VB et al. (2004) Corticotropin-releasing factor receptors CRF1 and CRF2 exert both additive and opposing influences on defensive startle behavior. J. Neurosci. 24: 6545-6552.
6. Wiley KE and Davenport AP (2004) CRF2 receptors are highly expressed in the human cardiovascular system and their cognate ligands urocortins 2 and 3 are potent vasodilators. Br. J. Pharmacol. 143: 508-514.
7. Zorrilla EP et al. (2004) Human urocortin 2, a corticotropin-releasing factor (CRF)2 agonist, and ovine CRF, a CRF1 agonist, differentially alter feeding and motor activity. J. Pharmacol. Exp. Ther. 310: 1027-1034.