

**PRODUCT DATASHEET**
**ChemiScreen™ PRP/GPR10 Prolactin-Releasing Peptide Membrane Preparation**

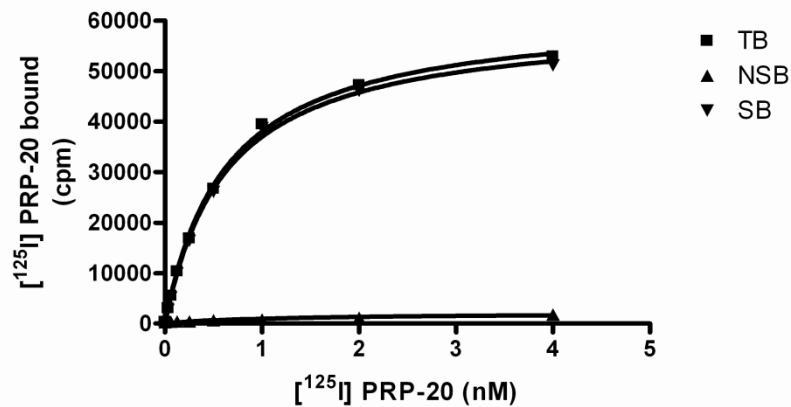
<b>CATALOG NUMBER:</b>	HTS057M	<b>QUANTITY:</b>	200 units
<b>LOT NUMBER:</b>		<b>VOLUME/CONCENTRATION</b>	1 mL, 1 mg/mL

**BACKGROUND**

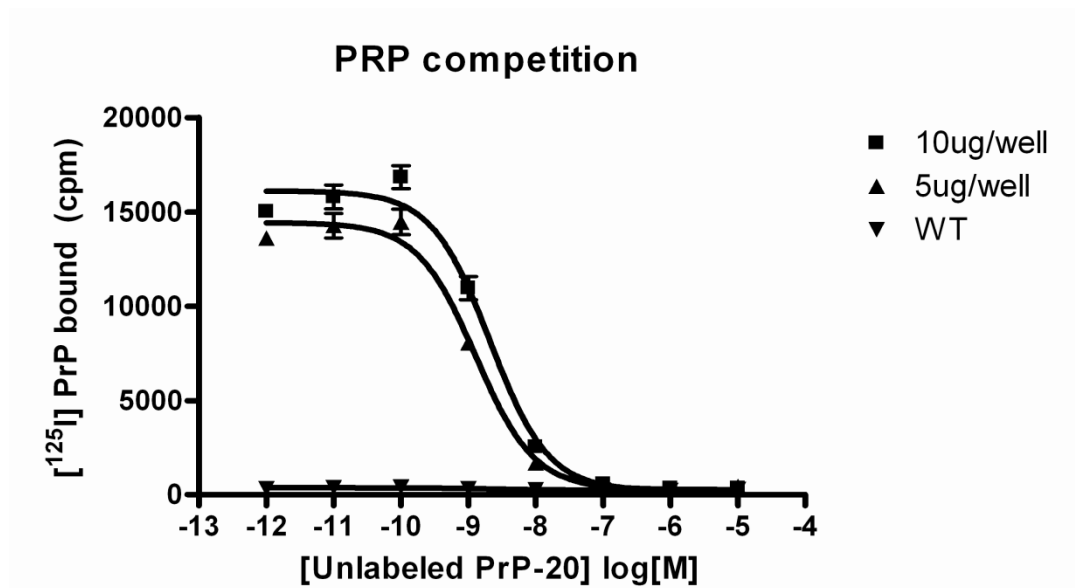
PRP, also known as GPR10 or hGR3, is a G<sub>q</sub>-coupled receptor for prolactin-releasing peptide that is expressed in the pituitary (Hinuma *et al.*, 1998). Genetic studies in rodents indicate that lack of GPR10 leads to hyperphagia, obesity and dyslipidemia (Gu *et al.*, 2004; Watanabe *et al.*, 2005). In humans, genetic variations in GPR10 are associated with lowered blood pressure (Bhattacharyya *et al.*, 2003). The GPR10/PRP 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 GPR10/PRP interactions with prolactin-releasing peptide. The membrane preparations exhibit a K<sub>d</sub> of 0.59-0.66 nM for [<sup>125</sup>I]-PRP-20. With 5 μg/well PRP Membrane Prep and 0.25 nM [<sup>125</sup>I]-PRP-20, a greater than 30-fold signal-to-background ratio was obtained.

**APPLICATIONS**

Radioligand binding assay



**Figure 1. Saturation binding for PRP.** 5 μg/well PRP Membrane Preparation was incubated with increasing amount of [<sup>125</sup>I]-PRP-20 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 1000-fold excess unlabeled PRP-20. Specific binding (SB) was determined by subtracting NSB from TB.



**Figure 2. Competition binding for PRP.** PRP Membrane Preparation (5 or 10  $\mu\text{g}/\text{well}$ ) or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.25 nM  $[^{125}\text{I}]$ -PRP-20 and increasing concentrations of unlabeled PRP-20, and more than 30-fold signal:background was obtained.

**SPECIFICATIONS:** 1 unit = 5  $\mu\text{g}$  membrane preparation  
 Bmax: 8.9pmol/mg  
 K<sub>d</sub>: 0.62 nM  
 Signal:Background: 30-fold

**TRANSFECTION:** Human Full-length human GPR10 cDNA encoding PRP (Accession Number: NM\_004248)

**HOST CELLS:** Chem-1, an adherent mammalian cell line without any endogenous GPR10 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 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<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.2% BSA, filtered and stored at 4°C

**Radioligand:**  $[^{125}\text{I}]$  PRP-20 (Perkin Elmer # NEX384)

**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 30-fold signal:background with  $[^{125}\text{I}]$ -labeled PRP-20 at 0.25 nM.

**PRESENTATION:**

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.

Packaging method: Membrane protein was adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

**STORAGE/HANDLING:** Store at  $-70^{\circ}\text{C}$ . Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw.

**REFERENCES:**

Bhattacharyya S *et al.* (2003) Association of polymorphisms in GPR10, the gene encoding the prolactin-releasing peptide receptor with blood pressure, but not obesity, in a U.K. Caucasian population. *Diabetes* 52: 1296-9.

Gu W *et al.* (2004) The prolactin-releasing peptide receptor (GPR10) regulates body weight homeostasis in mice. *J. Mol. Neurosci.* 22: 93-103.

Hinuma S *et al.* (1998) A prolactin-releasing peptide in the brain. *Nature* 393: 272-6.

Watanabe TK *et al.* (2005) Mutated G-protein-coupled receptor GPR10 is responsible for the hyperphagia/dyslipidaemia/obesity locus of Dmo1 in the OLETF rat. *Clin. Exp. Pharmacol. Physiol.* 32: 355-66.

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