

**CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN GPR103/QRFP NEUROPEPTIDE RECEPTOR**

CATALOG NUMBER:	HTS189M	QUANTITY:	200 units
LOT NUMBER:	R0708E0057	VOLUME/CONCENTRATION:	2 mL, 1 mg/mL
BACKGROUND:	GPR103 is a Gq-coupled receptor that is located in the brain and adrenal gland, and is activated by a 43-amino acid RFamide peptide, QRFP43, and a 26 amino acid fragment, QRFP26/26RFa/Peptide 518 (Jiang <i>et al.</i> , 2003; Fukusumi <i>et al.</i> , 2003). QRFP43 is the active form in rat brain, and intracerebral administration of QRFP43 induces feeding and metabolic rate (Takayasu <i>et al.</i> , 2006; Moriya <i>et al.</i> , 2006). Millipore's GPR103 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 GPR103. The membrane preparations exhibit a <i>Kd</i> of 0.63nM for [¹²⁵ I]-QRFP43. With 10 μ g/well GPR103 Membrane Prep and 0.35 nM [¹²⁵ I]- QRFP43, a greater than 6-fold signal-to-background ratio was obtained.		
APPLICATIONS:	Radioligand binding assay		

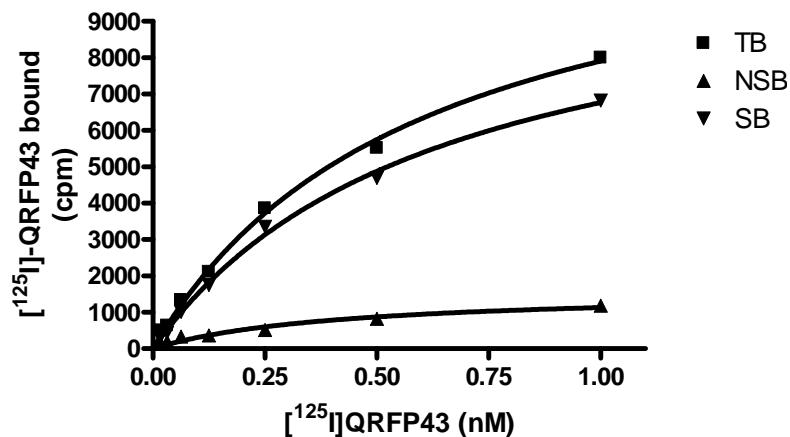


Figure 1. Saturation binding for GPR103. 10 μ g/well GPR103 Membrane Preparation was incubated with increasing amount of [¹²⁵I]QRFP43 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled 26Ffa human Hypothalamic Peptide. Specific binding (SB) was determined by subtracting NSB from TB.

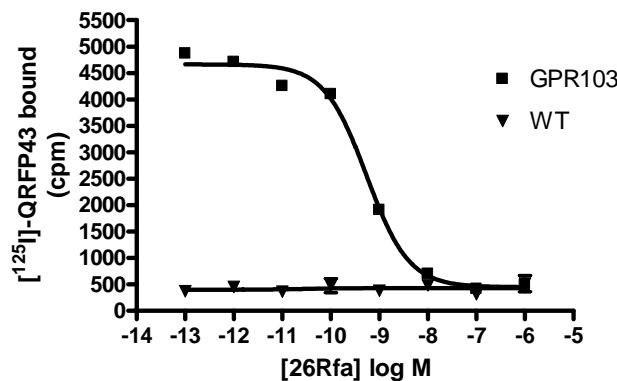


Figure 2. Competition binding for GPR103. 10 μ g/well GPR103 Membrane Preparation or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 0.35 nM [125 I]-QRFP43 and increasing concentrations of unlabeled 25Rfa, and more than 6- fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of GPR103 membrane prep.

	10 μ g/well
Signal:background	10
Specific binding (cpm)	4217.1

SPECIFICATIONS: 1 unit = 10 μ g membrane preparation

Bmax: 0.81 pmol/mg

K_d : 0.63 nM

Species: Human GPR103 (Accession Number: NM_198179)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous GPR103 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 MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

Radioligand: [125 I]QRFP43 (Perkin Elmer # NEX408)

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 6-fold signal:background with ^{125}I -labeled QRFP43 at 0.35 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:

Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.

REFERENCES:

Fukusumi S *et al.* (2003) A new peptidic ligand and its receptor regulating adrenal function in rats. *J. Biol. Chem.* 278: 46387-46395.

Jiang Y. *et al.* (2003) Identification and characterization of a novel RF-amide peptide ligand for orphan G-protein-coupled receptor SP9155. *J. Biol. Chem.* 278: 27652-27657.

Moriya R *et al.* (2006) RFamide peptide QRFP43 causes obesity with hyperphagia and reduced thermogenesis in mice. *Endocrinology* 147: 2916-2922.

Takayasu S *et al.* (2006) A neuropeptide ligand of the G protein-coupled receptor GPR103 regulates feeding, behavioral arousal, and blood pressure in mice. *Proc. Natl. Acad. Sci. USA* 103: 7438-7443.

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