

**CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN PTH1R PEPTIDE RECEPTOR**

CATALOG NUMBER:	HTS030M	QUANTITY:	200 units
LOT NUMBER:	0604028132	VOLUME/CONCENTRATION:	1 mL, 0.5 mg/mL

BACKGROUND: Parathyroid hormone (PTH) plays a critical role in mineral ion homeostasis, particularly in bone and kidney (Mannstadt et al., 1999; Gensure et al., 2005). A related peptide, parathyroid hormone-related peptide (PTHrP), plays an important role in skeletal development. Both peptides exert their biological actions by binding to a class B GPCR, PTH1R, that signals primarily by activation of adenylate cyclase (Schipani et al., 1993). Mutations in PTH1R have been identified as the cause of Jansen's metaphyseal chondrodysplasia, Blomstrand's chondrodysplasia, and enchondromatosis (Schipani and Provot, 2003). Intermittant treatment with PTH has important clinical utility in building bone mass in patients with osteoporosis (Rosen, 2003). Chemicon's cloned human PTH1R-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant PTH1R expression on the cell surface and contains high levels of the promiscuous G protein G α 15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between PTH1R and its ligands.

APPLICATIONS: Radioligand binding assay, and GTP γ S binding.

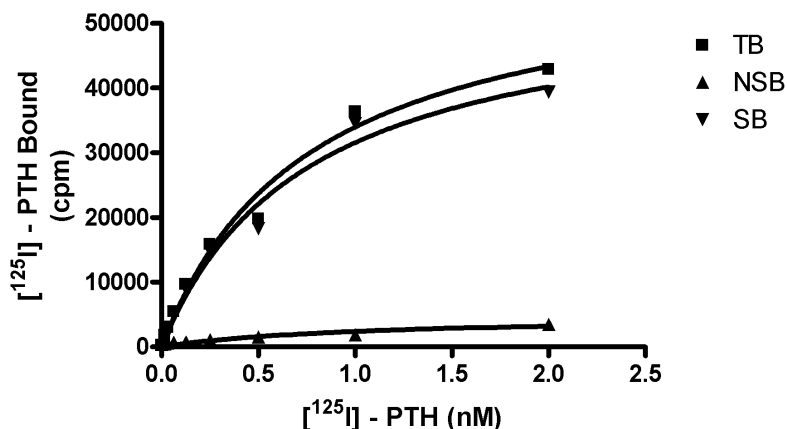


Figure 1. Saturation binding for PTH. 10 μ g/well PTH Membrane Preparation was incubated with increasing amount of ¹²⁵I-labeled PTH in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled PTH. Specific binding (SB) was determined by subtracting NSB from TB.

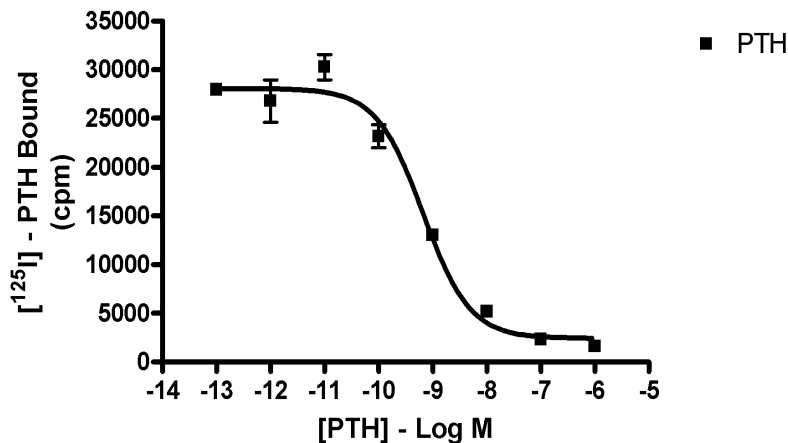


Figure 2. Competition binding for PTH. 5.0 µg/well PTH Membrane Preparation (HTS030M) was incubated with 0.75 nM ¹²⁵I-labeled PTH and increasing concentrations of unlabeled PTH, and more than 10-fold signal:background was obtained.

SPECIFICATIONS: Bmax: 2.66 pmol/mg; Kd: 0.75 nM
Species: Full-length human PTH1R cDNA (Accession Number: NM_000316)
HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous PTH 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 MgCl₂, 1 mM CaCl₂, 0.2% BSA, filtered and stored at 4°C

Radioligand: [¹²⁵I] PTH (Perkin Elmer#: NEX397)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl, 0.1% BSA, filtered and stored at 4°C.

One vial contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 5-fold signal:background with ¹²⁵I-labeled PTH at 0.75 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 0.5 mg/ml in 1 ml 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:

Gensure RC et al. (2005) Parathyroid hormone and parathyroid hormone-related peptide, and their receptors. *Biochem. Biophys. Res. Commun.* 328: 666-78

Mannstadt M et al. (1999) Receptors for PTH and PTHrP: their biological importance and functional properties. *Am. J. Physiol.* 277(5 Pt 2): F665-75.

Rosen CJ (2003) The cellular and clinical parameters of anabolic therapy for osteoporosis. *Crit. Rev. Eukaryot. Gene Expr.* 13: 25-38.

Schipani E and Provot S. (2003) PTHrP, PTH, and the PTH/PTHrP receptor in endochondral bone development. *Birth Defects Res. C. Embryo Today* 69: 352-62

Schipani E (1993) Identical complementary deoxyribonucleic acids encode a human renal and bone parathyroid hormone (PTH)/PTH-related peptide receptor. *Endocrinology* 132: 2157-65

For research use only; not for use as a diagnostic.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 μ L or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.