

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN TP PROSTANOID RECEPTOR

CATALOG NUMBER: HTS081M **QUANTITY:** 200 units
LOT NUMBER: **VOLUME/CONCENTRATION:** 1 mL, 2 mg/mL

BACKGROUND: TP receptors (Thromboxane A2 receptors) are widely distributed among different organ systems and have been localized on both cell membranes and intracellular structures. TP receptors belong to G protein-coupled receptor family (Hirata *et al.*, 1991). Activation of TP receptors induces platelet aggregation, vascular and respiratory smooth muscle constriction, and enhances mitogenic responses of vascular smooth muscle cells that are stimulated by growth factors (Ali *et al.*, 1993; Hanasaki *et al.*, 1990). The human TP receptor is encoded by a single gene that is alternatively spliced at the carboxyl terminus, resulting in two isoforms, TP α (343 residues) and TP β (407 residues). Both isoforms couple to Gq pathway, but couple oppositely to adenylate cyclase. The cDNA encoding human TP α has been stably expressed in the Chem-1 host, which supports high levels of recombinant receptor 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. The membrane preparations exhibit a Kd of 9.2 nM for [³H]-SQ 29548. With 10 nM [³H]-SQ 29548, one unit (10 μ g/well) TP Membrane Prep yields greater than 5-fold signal-to-background ratio.

APPLICATIONS: Radioligand binding assay

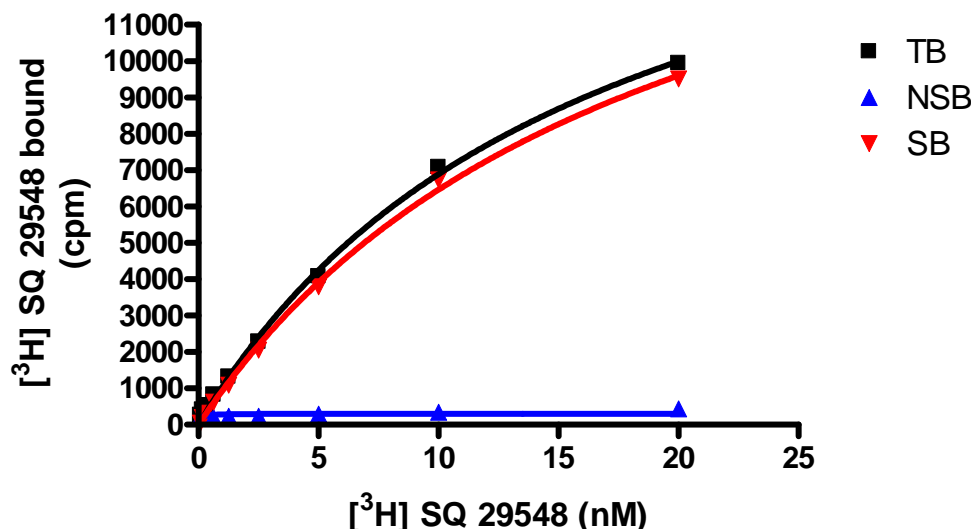


Figure 1. Saturation binding for TP. 10 μ g/well TP Membrane Preparation was incubated with increasing amount of [³H]-SQ 29548 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of more than 500-fold excess unlabeled ICI 192,605. Specific binding (SB) was determined by subtracting NSB from TB.

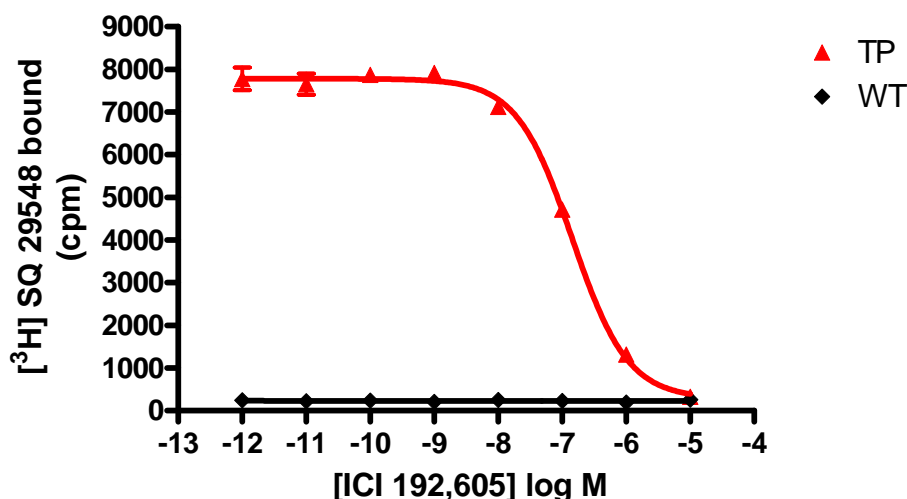


Figure 2. Competition binding for TP. TP Membrane Preparation (5 or 10 µg/well) or Wild-Type Chem-1 membrane preparation (WT; Millipore Catalog # HTS000MC1) was incubated with 15 nM [³H]-SQ 29548 and increasing concentrations of unlabeled ICI 192,605, and more than 5- fold signal:background was obtained.

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

	10 µg/well
Signal:background	22.7
Specific binding (cpm)	7431

SPECIFICATIONS: 1 unit = 10 µg membrane preparation
 B_{max}: 13.9 pmol/mg
 K_d: 14.6 nM

Species: Human Full-length human TBXA2R cDNA encoding the TP short form (TP α)
 (Accession Number: [NM_001060](#))

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous TP 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 50 mM 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: [³H]-SQ 29548 (Perkin Elmer # NET936)



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 5-fold signal:background with ³H-labeled SQ 29548 at 15 nM.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with 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:

Ali S *et al.* (1993) Thromboxane A2 stimulates vascular smooth muscle hypertrophy by unregulating the synthesis and release of endogenous basic fibroblast growth factor. *J. Biol. Chem.* 268: 17397–17403.

Hanasaki K *et al.* (1989) Biochemical characterization and comparison of rat thromboxane A2/prostaglandin H2 receptors in platelets and cultures aortic smooth muscle cells. *Biochem Pharmacol* 38: 2967–2976.

Hirata M *et al.* (1991) Cloning and expression of the cDNA for a human thromboxane A₂ receptor gene. *Nature* 349: 617-620.

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 µL 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.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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.

©2006 - 2012: EMD Millipore Corporation. All rights reserved. No part of these works may be reproduced in any form without permission in writing.