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CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN GPR109A RECEPTOR

CATALOG NUMBER:	HTS201M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

Nicotinic acid (niacin), a vitamin of the B complex, is used clinically in high doses to decrease total plasma levels of cholesterol. Interestingly, the total cholesterol levels and low-density lipoprotein (LDL) concentrations decrease, while the high-density lipoprotein (HDL) concentrations increase with nicotinic acid treatment. The cholesterol-lowering effects of nicotinic acid result from inhibition of lipolysis in adipose tissue, which decreases plasma levels of free fatty acid (FFA) (Altschul *et al.*, 1955; Carlson, 1963). In a study of nicotinic acid and myocardial infarction in the Coronary Drug Project (1966-1975), patients receiving 3 g/day nicotinic acid exhibited reduced rates of myocardial infarction (Coronary Drug Project Research Group, 1975). However, an unwanted effect of high doses of nicotinic acid is vasodilatation, occurring mainly in the upper body and face, known as flushing. Recently two receptors for nicotinic acid have been identified as class A G protein-coupled receptors that couple to G_i to inhibit accumulation of cAMP (Offermans, 2006). GPR109A (also known as HM74A in humans and PUMA-G in mice) is a high affinity receptor for nicotinic acid, whereas GPR109B (also known as HM74) is a low affinity receptor for nicotinic acid that is found in humans but not rodents (Wise *et al*., 2003). GPCR109A is found primarily in adipose tissue and immune cells. Millipore's GPR109A 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 GPR109A interactions with its ligands. The membrane preparations exhibit EC50s of 3.65 μM for nicotinic acid in a GTPγS binding assay.

APPLICATIONS:

GTPγS Binding and Radioligand Binding Assay.

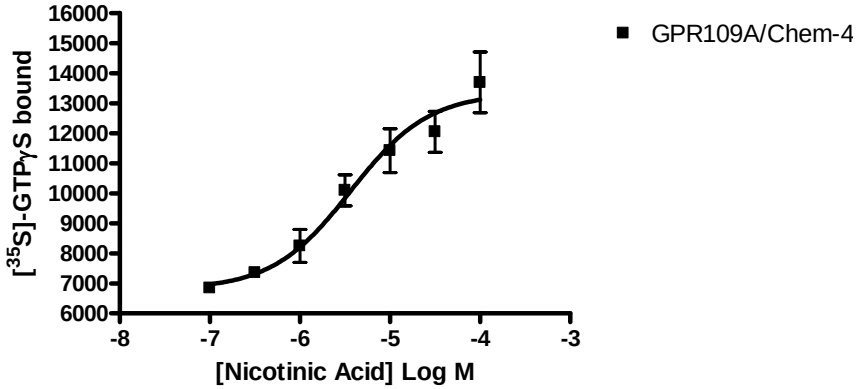

Figure 1. Binding of [^{35}S]-GTPγS to GPR109A membrane preparation. 5 μg/well GPR109A Membrane Preparation (catalog # HTS201M) was incubated with 0.3 nM [^{35}S]-GTPγS and increasing amounts of unlabeled nicotinic acid. Bound radioactivity was determined by filtration and scintillation counting.

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SPECIFICATIONS: 1 unit = 5 μg

EC50 in GTPγS binding assay by Nicotinic Acid: ~ 3.65 μM

Species: Full-length human GPR109A cDNA (Accession Number: NM_177551.3)

HOST CELLS: Chem-4, an adherent cell line expressing the promiscuous G-protein

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ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [^{35}S]-GTPγS (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM $MgCl_2$/0.5 μM GDP in a nonbinding 96-well plate. Unlabeled nicotinic acid was added to the final concentration indicated in Figure 1 (final volume 100 μL), and incubated for 30 min at 30°C. The binding reaction is transferred to a GF/B filter plate (Millipore MAHF B1H) previously prewetted with water, and washed 3 times (1 mL per well per wash) with cold 10 mM sodium phosphate, pH 7.4. The plate is dried and counted.

One vial contains enough membranes for at least 200 assays (units), where one unit is the amount of membrane that will yield greater than 1000 cpm specific nicotinic acid-stimulated [^{35}S]-GTPγS binding.

The GPR109A membrane preparation is expected to be functional in a radioligand binding assay; however, the end user will need to determine the optimal radiolabeled ligand for use with this product.

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 1 mg/ml in packaging buffer, rapidly frozen, and stored at -80^{o}C.

STORAGE/HANDLING:

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

REFERENCES:

Altschul R *et al*. (1955) Influence of nicotinic acid on serum cholesterol in man. *Arch. Biochem.* 54: 558-559.

Carlson LA (1963) Studies on the effect of nicotinic acid on catecholamine stimulated lipolysis in adipose tissue *in vitro*. *Acta Med. Scand*. 173: 719-722.

Coronary Drug Project Research Group (1975) Clofibrate and niacin in coronary heart disease. *J. Am. Med. Assoc.* 231: 360-381.

Offermans S (2006) The nicotinic acid receptor GPR109A (HM74A or PUMA-G) as a new therapeutic target. *Trends Pharmacol. Sci.* 27: 384-390.

Wise A *et al*. (2003) Molecular identification of high and low affinity receptors for nicotinic acid. *J. Biol. Chem.*278: 9869-9874.

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