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CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN GPR41 FREE FATTY ACID RECEPTOR

CATALOG NUMBER:	HTS135M	**QUANTITY:**	200 units
LOT NUMBER:	RI08010002	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

GPR41 is a GPCR that, along with GPR43, is activated by short chain carboxylic acids formate, acetate, proprionate, butyrate and pentanoate (Brown *et al*., 2003; Brown *et al*., 2005). Binding of these ligands to GPR41 selectively activates Gi to inhibit cAMP accumulation. Expression of GPR41 is prominent in adipose tissue, increases during differentiation of cultured adipocytes, and allows short chain carboxylic acids to stimulate leptin synthesis in adipocytes (Xiong *et al*., 2003). Millipore's GPR41 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 GPR41 interactions with its ligands. The cell line exhibits a calcium response with EC50s of 51.5µM for sodium propionate. The membrane preparations exhibit EC50s of 30.1 µM for sodium propionate in a GTPγS binding assay.

APPLICATIONS:

GTPγS Binding and Radioligand Binding Assay.

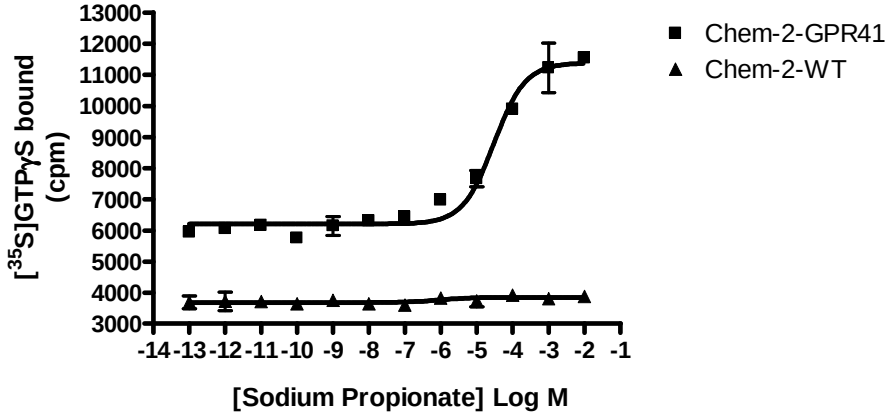

Figure 1. Binding of [^{35}S]-GTPγS to GPR41 membrane preparation. 5 µg/well GPR41 Membrane Preparation (catalog # HTS135M) was incubated with 0.3 nM [^{35}S]-GTPγS and increasing amounts of unlabeled sodium propionate. Bound radioactivity was determined by filtration and scintillation counting.

SPECIFICATIONS: 1 unit = 5 µg
EC50 in GTPγS binding assay by sodium propionate: ~ 30.1 µM

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Species: Full-length human GPR41 cDNA (Accession Number: NM_005304)

HOST CELLS: Chem-2, an suspension cell line expressing the promiscuous G-protein, Gα16.

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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 sodium propionate 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. The plate is washed 3 times (1 mL per well per wash) with cold 10 mM sodium phosphate, pH 7.4, then 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 sodium propionate-stimulated [^{35}S]-GTPγS binding.

The GPR41 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°C.

STORAGE/HANDLING:

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

REFERENCES:

Brown AJ *et al.* (2003) The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J. Biol. Chem.* 278: 11312-11319.

Brown AJ *et al.* (2005) A family of fatty acid binding receptors. *DNA Cell Biol.* 24: 54-61.

Xiong Y *et al.* (2004) Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proc. Natl. Acad. Sci. USA* 101: 1045-1050.

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