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CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN GPR43 FREE FATTY ACID RECEPTOR

CATALOG NUMBER:	HTS063M	**QUANTITY:**	200 units
LOT NUMBER:	RI08010003	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

GPR43 is a GPCR that, along with GPR41, is activated by short chain carboxylic acids formate, acetate, proprionate, butyrate and pentanoate. Neutrophils and other leukocytes selectively express GPR43, and GPR43 mediates calcium flux and chemotaxis in these cells by coupling to both G_q and G_i (Brown *et al*., 2003; Le Poul *et al*., 2003). Millipore's GPR43 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 GPR43 interactions with its ligands. The cell line exhibits a calcium response with EC50s of 11.8µM for sodium propionate. The membrane preparations exhibit EC50s of 40.7 µM for sodium propionate in a GTPγS binding assay.

APPLICATIONS:

GTPγS Binding and Radioligand Binding Assay.

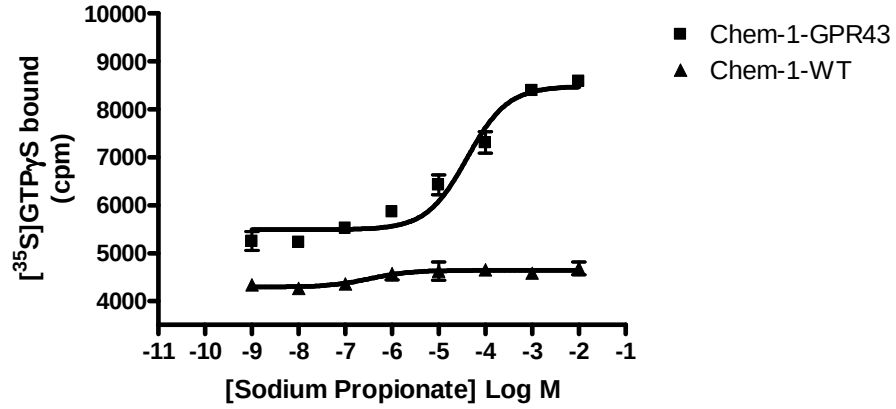

Figure 1. Binding of [^{35}S]-GTPγS to GPR43 membrane preparation. 5 µg/well GPR43 Membrane Preparation (catalog # HTS063M) was incubated with 0.3 nM [^{35}S]-GTPγS and increasing amounts of unlabeled sodium propionate. Bound radioactivity was determined by filtration and scintillation counting.

SPECIFICATIONS: 1 unit = 5 µg
EC50 in GTPγS binding assay by sodium propionate: ~ 40.7 µM

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Species: Full-length human GPR43 cDNA (Accession Number: BC096201)

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.
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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 GPR43 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:

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

Le Poul E *et al.* (2003) Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J. Biol. Chem.* 278: 25481-9

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