

HM74A siRNA (r): sc-270546

BACKGROUND

HM74, also known as PUMAG or Puma-g, is a member of the G protein-coupled receptor (GPCR) superfamily. In humans, HM74 is encoded by two different genes (GPR109A and GPR109B) that produce proteins, namely HM74A and HM74 (or HM74B), which are 96% homologous. In mice and rats, only one gene encodes the HM74 protein (Gpr109a). HM74 is a G_i protein-coupled receptor that mediates the metabolic effects of nicotinic acid. Localizing to the cell membrane, HM74 is highly expressed in adipocytes, immune cells and spleen. Like all members of the GPCR superfamily, HM74 contains seven transmembrane domains. HM74 lacks the N-linked glycosylation sites near the N-terminus that are present in other GPCR family members. Furthermore, HM74 shows a more diverged amino acid sequence homology from most family members, implying different ligand specificity.

REFERENCES

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PRODUCT

HM74A siRNA (r) is a pool of 2 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HM74A shRNA Plasmid (r): sc-270546-SH and HM74A shRNA (r) Lentiviral Particles: sc-270546-V as alternate gene silencing products.

For independent verification of HM74A (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270546A and sc-270546B.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

HM74A siRNA (r) is recommended for the inhibition of HM74A expression in rat cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

HM74 (D-8): sc-377292 is recommended as a control antibody for monitoring of HM74A gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HM74A gene expression knockdown using RT-PCR Primer: HM74A (r)-PR: sc-270546-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.