

AGS3 siRNA (h): sc-44441

BACKGROUND

Activators of G-protein signaling (AGS) are non-G protein-coupled receptor (GPCR)-ligand-induced initiators of heterotrimeric G-protein signaling pathways that function either downstream of GPCR effectors or at the level of heterotrimeric G-proteins. AGS3 is a G_{α_i} -binding protein that is capable of displacing $G_{\beta\gamma}$ and associating with G_{α} -GDP, thereby stabilizing the GDP-bound conformation of G_{α} . AGS3 localizes to the cytoplasm and is expressed in rat brain, PC12 cells, NG108-15 cells and DDT₁-MF2 smooth muscle cells. In rat, a 227 amino acid long form of AGS3 that contains seven TPR (tetratricopeptide repeat) domains, which target proteins to subcellular regions of neuroblasts, is more prevalent in adult rat brain, whereas the 166 amino acid short form of AGS3 is more prevalent in adult rat heart.

REFERENCES

1. Takesono, A., et al. 1999. Receptor-independent activators of heterotrimeric G protein signaling pathways. *J. Biol. Chem.* 274: 33202-33205.
2. Natochin, M., et al. 2000. AGS3 inhibits GDP dissociation from G_{α} subunits of the G_i family and rhodopsin-dependent activation of transducin. *J. Biol. Chem.* 275: 40981-40985.
3. De Vries, L., et al. 2000. Activator of G protein signaling 3 is a guanine dissociation inhibitor for G_{α_i} subunits. *Proc. Natl. Acad. Sci. USA* 97: 14364-14369.
4. Bernard, M.L., et al. 2001. Selective interaction of AGS3 with G proteins and the influence of AGS3 on the activation state of G proteins. *J. Biol. Chem.* 276: 1585-1593.

CHROMOSOMAL LOCATION

Genetic locus: GPSM1 (human) mapping to 9q34.3.

PRODUCT

AGS3 siRNA (h) is a pool of 3 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 AGS3 shRNA Plasmid (h): sc-44441-SH and AGS3 shRNA (h) Lentiviral Particles: sc-44441-V as alternate gene silencing products.

For independent verification of AGS3 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44441A, sc-44441B and sc-44441C.

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

AGS3 siRNA (h) is recommended for the inhibition of AGS3 expression in human 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

AGS3 (G-2): sc-271721 is recommended as a control antibody for monitoring of AGS3 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 AGS3 gene expression knockdown using RT-PCR Primer: AGS3 (h)-PR: sc-44441-PR (20 μ l, 466 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Vural, A., et al. 2016. Activator of G-protein signaling 3-induced lysosomal biogenesis limits macrophage intracellular bacterial infection. *J. Immunol.* 196: 846-856.

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.