SANTA CRUZ BIOTECHNOLOGY, INC.

AGA siRNA (h): sc-89013

BACKGROUND

AGA (aspartylglucosaminidase) is a 346 amino acid precursor protein that belongs to the Ntn-hydrolase family and is cleaved to produce an α chain and a β chain. Localized to the lysosome, AGA functions as a heterotetramer composed of two α and two β chains that work together to cleave the GlcNAc-Asn bond that joins oligosaccharides to target glycoproteins. Defects in the gene encoding AGA are the cause of aspartylglucosaminuria (AGU), a lysosomal storage disease that is characterized by severe mental retardation and mild connective tissue abnormalities. The gene encoding AGA maps to human chromosome 4, which encodes nearly 6% of the human genome and has the largest gene deserts (regions of the genome with no protein encoding genes) of all of the human chromosomes.

REFERENCES

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3. Saarela, J., et al. 2001. Molecular pathogenesis of a disease: structural consequences of aspartylglucosaminuria mutations. Hum. Mol. Genet. 10: 983-995.
4. Saarela, J., et al. 2004. Autoproteolytic activation of human aspartylglucosaminidase. Biochem. J. 378: 363-371.
5. Saarela, J., et al. 2004. A novel aspartylglucosaminuria mutation affects translocation of aspartylglucosaminidase. Hum. Mutat. 24: 350-351.
6. Jackson, M., et al. 2005. Elevation of plasma aspartylglucosaminidase is a useful marker for the congenital disorders of glycosylation type I (CDG I). J. Inherit. Metab. Dis. 28: 1197-1198.
7. Saito, S., et al. 2008. Structural basis of aspartylglucosaminuria. Biochem. Biophys. Res. Commun. 377: 1168-1172.
8. Michelakakis, H., et al. 2009. Plasma lysosomal enzyme activities in congenital disorders of glycosylation, galactosemia and fructosemia. Clin. Chim. Acta 401: 81-83.

CHROMOSOMAL LOCATION

Genetic locus: AGA (human) mapping to 4q34.3.

PRODUCT

AGA 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AGA shRNA Plasmid (h): sc-89013-SH and AGA shRNA (h) Lentiviral Particles: sc-89013-V as alternate gene silencing products.

For independent verification of AGA (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-89013A, sc-89013B and sc-89013C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

AGA siRNA (h) is recommended for the inhibition of AGA 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 µM in 66 µl. 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

AGA (H-8): sc-514075 is recommended as a control antibody for monitoring of AGA 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 AGA gene expression knockdown using RT-PCR Primer: AGA (h)-PR: sc-89013-PR (20 µl). 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.