Afaf siRNA (h): sc-92976



The Power to Question

BACKGROUND

Afaf (acrosome formation-associated factor), also known as C9orf11, is a 294 amino acid single-pass type I membrane protein. Existing as two alternatively spliced isoforms, Afaf isoform 1 is highly expressed in testis, while isoform 2 is expressed at low levels in skin and blood. Encoded by a gene that maps to human chromosome 9p21.2, Afaf localizes in inner and outer membranes of developing acrosomes and participates in acrosome biogenesis. Involved in calcium-triggered acrosome exocytosis, Afaf operates upstream of calcium flow from within the acrosome. Afaf is also linked to endocytic pathways through its down-regulation of transferrin endocytosis in HeLa cells. Afaf may play an important role in membrane trafficking during acrosome formation and may participate in fertilization. Afaf might also interact with SNAP 25, an integral element in both exocytosis and endosomal trafficking.

REFERENCES

- Li, Y.C., Hu, X.Q., Zhang, K.Y., Guo, J., Hu, Z.Y., Tao, S.X., Xiao, L.J., Wang, Q.Z., Han, C.S. and Liu, Y.X. 2006. Afaf, a novel vesicle membrane protein, is related to acrosome formation in murine testis. FEBS Lett. 580: 4266-4273.
- Shi, Y.Q., Li, Y.C., Hu, X.Q., Liu, T., Liao, S.Y., Guo, J., Huang, L., Hu, Z.Y., Tang, A.Y., Lee, K.F., Yeung, W.S., Han, C.S. and Liu, Y.X. 2009. Male germ cell-specific protein Trs4 binds to multiple proteins. Biochem. Biophys. Res. Commun. 388: 583-588.
- Oliveira, C.A., Victor-Costa, A.B. and Hess, R.A. 2009. Cellular and regional distributions of ubiquitin-proteasome and endocytotic pathway components in the epithelium of rat efferent ductules and initial segment of the epididymis. J. Androl. 30: 590-601.
- 4. Berruti, G., Ripolone, M. and Ceriani, M. 2010. USP8, a regulator of endosomal sorting, is involved in mouse acrosome biogenesis through interaction with the spermatid ESCRT-0 complex and microtubules. Biol. Reprod. 82: 930-939.
- 5. Hu, X.Q., Ji, S.Y., Li, Y.C., Fan, C.H., Cai, H., Yang, J.L., Zhang, C.P., Chen, M., Pan, Z.F., Hu, Z.Y., Gao, F. and Liu, Y.X. 2010. Acrosome formation-associated factor is involved in fertilization. Fertil. Steril. 93: 1482-1492.
- 6. Cai, H., Ren, Y., Li, X.X., Yang, J.L., Zhang, C.P., Chen, M., Fan, C.H., Hu, X.Q., Hu, Z.Y., Gao, F. and Liu, Y.X. 2010. Scrotal heat stress causes a transient alteration in tight junctions and induction of TGF-β expression. Int. J. Androl. 34: 352-362.
- Hermo, L., Pelletier, R.M., Cyr, D.G. and Smith, C.E. 2010. Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells. Part 2: changes in spermatid organelles associated with development of spermatozoa. Microsc. Res. Tech. 73: 279-319.

CHROMOSOMAL LOCATION

Genetic locus: EQTN (human) mapping to 9p21.2.

PROTOCOLS

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

PRODUCT

Afaf 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 Afaf shRNA Plasmid (h): sc-92976-SH and Afaf shRNA (h) Lentiviral Particles: sc-92976-V as alternate gene silencing products.

For independent verification of Afaf (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-92976A, sc-92976B and sc-92976C.

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

Afaf siRNA (h) is recommended for the inhibition of Afaf 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Afaf gene expression knockdown using RT-PCR Primer: Afaf (h)-PR: sc-92976-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.

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