SANTA CRUZ BIOTECHNOLOGY, INC.

ALF siRNA (h): sc-72482

BACKGROUND

ALF (TFIIA-α/β-like factor), also known as GTF2A1L (general transcription factor IIA 1-like), is a nuclear and cytoplasmic protein that is specifically expressed in haploid spermatids in the testis. ALF is believed to play a role in spermatogenesis, functioning as a transcription factor, and is related to the large α/β subunit of TFIIA, existing as the germ cell-specific paralog. Similar to TFIIA-α/β, ALF associates with the smaller TFIIA subunit, TFIIA-γ, and functions to stabilize the interaction between TBP and DNA by binding directly to TBP and the DNA (at the TATA box), thus forming a TBP/ALF/TATA complex which mediates the transcriptional output of a gene. Low expression levels of ALF have been associated with abnormal spermatogenesis and male infertility, further implying a role for ALF in spermatogenic cell function. The transcriptional expression of ALF appears to be regulated by DNA methylation.

REFERENCES

1. Upadhyaya, A.B., et al. 1999. Identification of a general transcription factor TFIIA-α/β homolog selectively expressed in testis. J. Biol. Chem. 274: 18040-18048.
2. Han, S.Y., et al. 2001. TFIIA-α/β-like factor is encoded by a germ cell-specific gene whose expression is upregulated with other general transcription factors during spermatogenesis in the mouse. Biol. Reprod. 64: 507-517.
3. Xie, W., et al. 2002. Regulation of ALF gene expression in somatic and male germ line tissues involves partial and site-specific patterns of methylation. J. Biol. Chem. 277: 17765-17774.
4. Han, S., et al. 2004. A short core promoter drives expression of the ALF transcription factor in reproductive tissues of male and female mice. Biol. Reprod. 71: 933-941.
5. Catena, R., et al. 2005. Proteolytic cleavage of ALF into α and β subunits that form homologous and heterologous complexes with somatic TFIIA and TRF2 in male germ cells. FEBS Lett. 579: 3401-3410.

CHROMOSOMAL LOCATION

Genetic locus: GTF2A1L (human) mapping to 2p16.3.

PRODUCT

ALF 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 ALF shRNA Plasmid (h): sc-72482-SH and ALF shRNA (h) Lentiviral Particles: sc-72482-V as alternate gene silencing products.

For independent verification of ALF (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-72482A, sc-72482B and sc-72482C.

PROTOCOLS

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

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

ALF siRNA (h) is recommended for the inhibition of ALF 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

ALF (24): sc-130304 is recommended as a control antibody for monitoring of ALF 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 ALF gene expression knockdown using RT-PCR Primer: ALF (h)-PR: sc-72482-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.