

# ALY siRNA (m): sc-38248

## BACKGROUND

ALY (also designated THO complex subunit 4, THOC4, REF1, Refbp1 and BEF) is the mammalian homolog of the yeast mRNA export factor Yralp. A transcriptional coactivator, ALY belongs to the cytidyltransferase family and is important in mRNA processing and export. During spliceosome assembly, it is recruited to messenger ribonucleoprotein (mRNP) complexes and becomes tightly associated with the spliced mRNP. Consistent with splicing-dependent recruitment, ALY co-localizes with splicing factors in the nucleus. It promotes transcriptional activation by promoting the dimerization of transcription factors containing basic leucine zipper domains. Although ubiquitously expressed, ALY specifically associates with the activation domains of LEF-1 and AML-1, both of which are protein components of the TCR  $\alpha$  enhancer complex. Research indicates that ALY may mediate context-dependent transcriptional activation by facilitating the functional collaboration of multiple proteins in the TCR  $\alpha$  enhancer complex.

## REFERENCES

1. Bruhn, L., et al. 1997. ALY, a context-dependent coactivator of LEF-1 and AML-1, is required for TCR  $\alpha$  enhancer function. *Genes Dev.* 11: 640-653.
2. Virbasius, C.M., et al. 1999. A human nuclear-localized chaperone that regulates dimerization, DNA binding, and transcriptional activity of bZIP proteins. *Mol. Cell* 4: 219-228.
3. Strasser, K., et al. 2000. Yra1p, a conserved nuclear RNA-binding protein, interacts directly with Mex67p and is required for mRNA export. *EMBO J.* 19: 410-420.
4. Stutz, F., et al. 2000. REF, an evolutionary conserved family of hnRNP-like proteins, interacts with TAP/Mex67p and participates in mRNA nuclear export. *RNA* 6: 638-650.
5. Zhou, Z., et al. 2000. The protein ALY links pre-messenger-RNA splicing to nuclear export in metazoans. *Nature* 407: 401-405.
6. Luo, M.L., et al. 2001. Pre-mRNA splicing and mRNA export linked by direct interactions between UAP56 and ALY. *Nature* 413: 644-647.
7. Gatfield, D., et al. 2002. REF1/ALY and the additional exon junction complex proteins are dispensable for nuclear mRNA export. *J. Cell Biol.* 159: 579-588.

## CHROMOSOMAL LOCATION

Genetic locus: Alyref (mouse) mapping to 11 E2.

## PRODUCT

ALY siRNA (m) 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ALY shRNA Plasmid (m): sc-38248-SH and ALY shRNA (m) Lentiviral Particles: sc-38248-V as alternate gene silencing products.

For independent verification of ALY (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-38248A, sc-38248B and sc-38248C.

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

## APPLICATIONS

ALY siRNA (m) is recommended for the inhibition of ALY expression in mouse 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  $\mu$ M in 66  $\mu$ 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

ALY (11G5): sc-32311 is recommended as a control antibody for monitoring of ALY 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ALY gene expression knockdown using RT-PCR Primer: ALY (m)-PR: sc-38248-PR (20  $\mu$ 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](http://www.scbt.com) for detailed protocols and support products.