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ALY shRNA (h) Lentiviral Particles: sc-45390-V

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 α enhancer complex. Research indicates that ALY may mediate context-dependent transcriptional activation by facilitating the functional collaboration of multiple proteins in the TCR α enhancer complex.

REFERENCES

1. Bruhn, L., et al. 1997. ALY, a context-dependent coactivator of LEF-1 and AML-1, is required for TCR α 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.

CHROMOSOMAL LOCATION

Genetic locus: THOC4 (human) mapping to 17q25.3.

PRODUCT

ALY shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ALY siRNA (h): sc-45390 and ALY shRNA Plasmid (h): sc-45390-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

ALY shRNA (h) Lentiviral Particles is recommended for the inhibition of ALY expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ALY gene expression knockdown using RT-PCR Primer: ALY (h)-PR: sc-45390-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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