SANTA CRUZ BIOTECHNOLOGY, INC.

Alix siRNA (m): sc-60150

BACKGROUND

ALG-2-interacting protein (Alix), also designated programmed cell death 6-interacting protein (PDCD6-interacting protein and Hp95), is a cytoplasmic protein that interacts with apoptosis-associated proteins (ALG-2 and PDCD6) and with the endocytosis-regulator CIN85. Additionally, Alix interacts with the endosomal sorting complexes required for transport (ESCRT) proteins (tsg 101 and CHMP4) and can associate with HIV-1. The endophilins (SH3P4, SH3P8 and SH3P13), enzymes that change curvature of the membrane that are required for early and late steps of coated vesicle formation, also bind to Alix. Alix is involved in the concentration and sorting of cargo proteins of the multivesicular body for incorpoation into vesicles.

REFERENCES

1. Segura-Morales, C., et al. 2005. Tsg 101 and Alix interact with murine leukemia virus Gag and cooperate with NEDD4 ubiquitin ligases during budding. J. Biol. Chem. 280: 27004-27012.
2. Katoh, K., et al. 2005. The penta-EF-hand protein ALG-2 interacts directly with the ESCRT-I component tsg 101, and Ca^{2+}-dependently co-localizes to aberrant endosomes with dominant-negative AAA ATPase SKD1/VPS4B. Biochem. J. 391: 677-685.
3. Sakaguchi, T., et al. 2005. AIP1/Alix is a binding partner of Sendai virus C protein and facilitates virus budding. J. Virol. 79: 8933-8941.
4. Le Blanc, I., et al. 2005. Endosome-to-cytosol transport of viral nucleocapsids. Nat. Cell Biol. 7: 653-664.
5. Kim, J., et al. 2005. Structural basis for endosomal targeting by the Bro1 domain. Dev. Cell 8: 937-947.

CHROMOSOMAL LOCATION

Genetic locus: Pdcd6ip (mouse) mapping to 9 F3.

PRODUCT

Alix 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Alix shRNA Plasmid (m): sc-60150-SH and Alix shRNA (m) Lentiviral Particles: sc-60150-V as alternate gene silencing products.

For independent verification of Alix (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-60150A, sc-60150B and sc-60150C.

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

Alix siRNA (m) is recommended for the inhibition of Alix 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 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

Alix (1A12): sc-53540 is recommended as a control antibody for monitoring of Alix 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 Alix gene expression knockdown using RT-PCR Primer: Alix (m)-PR: sc-60150-PR (20 µl, 417 bp). 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.