

KAP3 siRNA (m): sc-40722

BACKGROUND

The mouse kinesin superfamily-associated protein 3 (KAP3) and the human homolog KAP3A are globular proteins that function as linkers of chromosome associated proteins. The mouse gene encoding KAP3 generates an additional alternative isoform, from the insertion of a stop codon at the C-terminus, to produce a truncated KAP3 protein designated KAP3B. KAP3 was originally shown to associate with kinesin superfamily proteins, KIF3A and KIF3B, which function as an axonal motor for membranous organelle transport. The initial studies involving the human homolog of KAP3, also designated SMAP (for Small G protein GDP dissociation stimulator (Smg GDS)-associated protein), indicated that KAP3 is an adaptor protein for Smg GDS and kinesin II and a kinase substrate for tyrosine phosphorylation by v-Src. Subsequent studies have shown that SMAP/KAP3A forms ternary complexes with HCAP (human chromosome-associated polypeptide), a member of the stability of mini-chromosomes family, and KIF3A/B. Once formed, these complexes assist in the association of chromosomes with the spindle and in chromosome movement during interphase.

REFERENCES

1. Yamazaki, H., et al. 1995. KIF3A/B: a heterodimeric kinesin superfamily protein that works as a microtubule plus end-directed motor for membrane organelle transport. *J. Cell Biol.* 130: 1387-1399.
2. Henson, J.H., et al. 1995. Immunolocalization of the heterotrimeric kinesin-related protein KRP(85/95) in the mitotic apparatus of sea urchin embryos. *Dev. Biol.* 171: 182-194.
3. Shimizu, K., et al. 1996. SMAP, an Smg GDS-associating protein having arm repeats and phosphorylated by Src tyrosine kinase. *J. Biol. Chem.* 271: 27013-27017.
4. Yamazaki, H., et al. 1996. Cloning and characterization of KAP3: a novel kinesin superfamily-associated protein of KIF3A/3B. *Proc. Natl. Acad. Sci. USA* 93: 8443-8448.
5. Shimizu, K., et al. 1998. Complex formation of SMAP/KAP3, a KIF3A/B ATPase motor-associated protein, with a human chromosome-associated polypeptide. *J. Biol. Chem.* 273: 6591-6594.

CHROMOSOMAL LOCATION

Genetic locus: Kifap3 (mouse) mapping to 1 H2.1.

PRODUCT

KAP3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see KAP3 shRNA Plasmid (m): sc-40722-SH and KAP3 shRNA (m) Lentiviral Particles: sc-40722-V as alternate gene silencing products.

For independent verification of KAP3 (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-40722A, sc-40722B and sc-40722C.

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

KAP3 siRNA (m) is recommended for the inhibition of KAP3 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 μ M in 66 μ 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

KAP3 (D-6): sc-55598 is recommended as a control antibody for monitoring of KAP3 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 κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 KAP3 gene expression knockdown using RT-PCR Primer: KAP3 (m)-PR: sc-40722-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.

PROTOCOLS

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