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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

ARK-2 siRNA (m): sc-43532

BACKGROUND

Aurora related kinase-1 (ARK-1, STK15, Aurora2, Aik1) and -2 (ARK-2, STK12, Aurora1) are centrosome-associated serine/threonine kinases that regulate centrosome separation, bipolar spindle assembly and chromosome segregation during mitosis. ARK-1 and -2 are expressed in the nucleus and localize to distinct portions of mitotic machinery such as the centrosome, spindle poles (ARK-1) and midbody (ARK-2) during mitosis. ARK-1 and -2 transcripts are present at high levels in human thymus and fetal liver. ARK-1 protein has elevated expression in colon carcinoma lines (HT-29, SNU-C2B, COLO 205, SW480, 837 and 948) and accumulates during metaphase in HeLa cells. ARK-2 protein levels are maximal during both S and G₂/M phases, whereas ARK-1 protein is degraded after G₂/M via the ubiquitin-proteasome pathway. ARK-2 has a unique genetic locus relative to ARK-1, suggesting that these two kinases, with oncogenic potential, have different roles in cell cycle progression.

REFERENCES

1. Bischoff, J.R., et al. 1998. A homologue of *Drosophila* aurora kinase is oncogenic and amplified in human colorectal cancers. *EMBO J.* 17: 3052-3065.
2. Zhou, H., et al. 1998. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. *Nat. Genetics* 20: 189-193.
3. Shindo, M., et al. 1998. cDNA cloning, expression, subcellular localization, and chromosomal assignment of mammalian aurora homologues, aurora-related kinase (ARK) 1 and 2. *Biochem. Biophys. Res. Commun.* 244: 285-292.
4. Kimura, M., et al. 1998. Identification and characterization of STK12/Aik2: a human gene related to aurora of *Drosophila* and yeast IPL1. *Cytogenet. Cell Genet.* 82: 147-152.
5. Giet, R. and Prigent, C. 1999. Aurora/Ipl1p-related kinases, a new oncogenic family of mitotic serine-threonine kinases. *J. Cell. Sci.* 112: 3591-3601.
6. Farruggio, D.C., et al. 1999. Cdc20 associates with the kinase Proc. *Natl. Acad. Sci. USA* 96: 7306-7311.

CHROMOSOMAL LOCATION

Genetic locus: Aurkb (mouse) mapping to 11 B3.

PRODUCT

ARK-2 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 ARK-2 shRNA Plasmid (m): sc-43532-SH and ARK-2 shRNA (m) Lentiviral Particles: sc-43532-V as alternate gene silencing products.

For independent verification of ARK-2 (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-43532A, sc-43532B and sc-43532C.

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

ARK-2 siRNA (m) is recommended for the inhibition of ARK-2 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

ARK-2 (A-3): sc-393357 is recommended as a control antibody for monitoring of ARK-2 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 ARK-2 gene expression knockdown using RT-PCR Primer: ARK-2 (m)-PR: sc-43532-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.