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ARK-2 shRNA (m) Lentiviral Particles: sc-43532-V

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 loci 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.

CHROMOSOMAL LOCATION

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

PRODUCT

ARK-2 shRNA (m) 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 µl frozen stock containing 1.0 x 10⁶ 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 ARK-2 siRNA (m): sc-43532 and ARK-2 shRNA Plasmid (m): sc-43532-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.

PROTOCOLS

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

APPLICATIONS

ARK-2 shRNA (m) Lentiviral Particles is recommended for the inhibition of ARK-2 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

ARK-2 (H-75): sc-25426 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.