SANTA CRUZ BIOTECHNOLOGY, INC.

AAK1 siRNA (h): sc-94850

BACKGROUND

Adaptor-related protein complex 2 (AP-2 complexes) operates during receptor-mediated endocytosis to initiate clathrin assembly, associates with membrane-bound receptors and mobilizes encodytic accessory factors. AAK1 (AP2 associated kinase 1), also known as adaptor-associated kinase 1, is a 961 amino acid protein belonging to the protein kinase superfamily and the serine/threonine protein kinase family. Encoded by a gene that maps to human chromosome 2p13.3, AAK1 participates in ATP and nucleotide binding, protein serine/threonine kinase functions and transferase activity. AAK1 phosphorylates a subunit of the AP-2 complex, which leads to binding of AP-2 to sorting signals in membrane-bound receptors, resulting in receptor endocytosis. AAK1 contains one protein kinase domain, with kinase activity stimulated by clathrin, and exists as two alternatively spliced isoforms.

REFERENCES

1. Ricotta, D., et al. 2002. Phosphorylation of the AP2 μ subunit by AAK1 mediates high affinity binding to membrane protein sorting signals. J. Cell Biol. 156: 791-795.
2. Conner, S.D., et al. 2002. Identification of an adaptor-associated kinase, AAK1, as a regulator of clathrin-mediated endocytosis. J. Cell Biol. 156: 921-929.
3. Korolchuk, V., et al. 2003. Kinases in clathrin-mediated endocytosis. Biochem. Soc. Trans. 31: 857-860.
4. Smythe, E., et al. 2003. The Ark1/Prk1 family of protein kinases. Regulators of endocytosis and the actin skeleton. EMBO Rep. 4: 246-251.
5. Conner, S.D., et al. 2003. Differential requirements for AP-2 in clathrin-mediated endocytosis. J. Cell Biol. 162: 773-779.
6. Zhang, L., et al. 2004. The serine/threonine kinase cyclin G-associated kinase regulates epidermal growth factor receptor signaling. Proc. Natl. Acad. Sci. USA 101: 10296-10301.
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CHROMOSOMAL LOCATION

Genetic locus: AAK1 (human) mapping to 2p13.3.

PRODUCT

AAK1 siRNA (h) 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 AAK1 shRNA Plasmid (h): sc-94850-SH and AAK1 shRNA (h) Lentiviral Particles: sc-94850-V as alternate gene silencing products.

For independent verification of AAK1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-94850A, sc-94850B and sc-94850C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

AAK1 siRNA (h) is recommended for the inhibition of AAK1 expression in human 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

AAK1 (LW-M11): sc-134242 is recommended as a control antibody for monitoring of AAK1 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 AAK1 gene expression knockdown using RT-PCR Primer: AAK1 (h)-PR: sc-94850-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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