

Eg5 siRNA (h): sc-37052

BACKGROUND

Eukaryotes contain a superfamily of microtubule-based motor proteins comprising kinesin and a number of related proteins that are thought to participate in various forms of intracellular motility, including cell division and organelle transport. KIF11 (also known as kinesin family member 11, Eg5 or TRIP5) is a slow, plus-end-directed microtubule-based motor of the BimC kinesin family that is essential for bipolar spindle formation during eukaryotic cell division. When the expression of KIF11 is blocked, centrosome migration halts and cells are arrested in mitosis with monoastrial microtubule arrays. KIF11 is phosphorylated on serine during S phase and on both serine and Thr 927 during mitosis, which regulates the association of Eg5 with the spindle apparatus (probably during early prophase). KIF11 is also known to be a member of the thyroid receptor interacting protein (Trip) family, and interacts with the thyroid hormone receptor only in the presence of thyroid hormone.

REFERENCES

1. Blangy, A., et al. 1995. Phosphorylation by p34^{cdc2} regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation *in vivo*. *Cell* 83: 1159-1169.
2. Lee, J.W., et al. 1995. Two classes of proteins dependent on either the presence or absence of thyroid hormone for interaction with the thyroid hormone receptor. *Mol. Endocrinol.* 9: 243-254.
3. Nakagawa, T., et al. 1997. Identification and classification of 16 new kinesin superfamily (KIF) proteins in mouse genome. *Proc. Natl. Acad. Sci. USA* 94: 9654-9659.
4. Whitehead, C.M., et al. 1998. Expanding the role of HsEg5 within the mitotic and post-mitotic phases of the cell cycle. *J. Cell Sci.* 111: 2551-2561.
5. Ferhat, L., et al. 1998. Expression of the mitotic motor protein Eg5 in post-mitotic neurons: implications for neuronal development. *J. Neurosci.* 18: 7822-7835.

CHROMOSOMAL LOCATION

Genetic locus: KIF11 (human) mapping to 10q23.33.

PRODUCT

Eg5 siRNA (h) is a target-specific 19-25 nt siRNA 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 Eg5 shRNA Plasmid (h): sc-37052-SH and Eg5 shRNA (h) Lentiviral Particles: sc-37052-V as alternate gene silencing products.

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

Eg5 siRNA (h) is recommended for the inhibition of Eg5 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 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

Eg5 (F-1): sc-374212 is recommended as a control antibody for monitoring of Eg5 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 Eg5 gene expression knockdown using RT-PCR Primer: Eg5 (h)-PR: sc-37052-PR (20 μ l, 593 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Even-Ram, S., et al. 2007. Myosin IIA regulates cell motility and actomyosin-microtubule crosstalk. *Nat. Cell Biol.* 9: 299-309.
2. Wang, G., et al. 2010. Nucleophosmin/B23 inhibits Eg5-mediated microtubule depolymerization by inactivating its ATPase activity. *J. Biol. Chem.* 285: 19060-19067.

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.