

CLASP2 siRNA (m): sc-142361

BACKGROUND

CLASP2 (cytoplasmic linker associated protein 2), also known as KIAA0627 or hOrbit2, is a 1,294 amino acid protein that contains five HEAT repeats and localizes to the cytoplasm and the cytoskeleton, as well as to the kinetochore and the Golgi apparatus. Expressed primarily in brain tissue, CLASP2 functions as a microtubule plus-end tracking protein that regulates the stability of dynamic microtubules and is required for the proper polarization of cytoplasmic microtubule arrays in migrating cells. CLASP2 interacts with EB1, EB3, ELKS and CLIP-115 and, in addition to stabilizing microtubules, plays an important role in maintaining the stability of the kinetochore and is crucial for proper chromosomal alignment. CLASP2 is subject to phosphorylation by GSK-3 β , an event that is thought to negatively regulate the ability of CLASP2 to bind to microtubules. Two isoforms of CLASP2, designated β and γ , exist due to alternative splicing events.

REFERENCES

1. Akhmanova, A., et al. 2001. Clasps are CLIP-115 and -170 associating proteins involved in the regional regulation of microtubule dynamics in motile fibroblasts. *Cell* 104: 923-935.
2. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 605853. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Lee, H., et al. 2004. The microtubule plus end tracking protein Orbit/MAST/CLASP acts downstream of the tyrosine kinase Abl in mediating axon guidance. *Neuron* 42: 913-926.
4. Mimori-Kiyosue, Y., et al. 2005. CLASP1 and CLASP2 bind to EB1 and regulate microtubule plus-end dynamics at the cell cortex. *J. Cell Biol.* 168: 141-153.
5. Wittmann, T., et al. 2005. Spatial regulation of CLASP affinity for microtubules by Rac1 and GSK3 β in migrating epithelial cells. *J. Cell Biol.* 169: 929-939.

CHROMOSOMAL LOCATION

Genetic locus: Clasp2 (mouse) mapping to 9 F3.

PRODUCT

CLASP2 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 CLASP2 shRNA Plasmid (m): sc-142361-SH and CLASP2 shRNA (m) Lentiviral Particles: sc-142361-V as alternate gene silencing products.

For independent verification of CLASP2 (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-142361A, sc-142361B and sc-142361C.

PROTOCOLS

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

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

CLASP2 siRNA (m) is recommended for the inhibition of CLASP2 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

CLASP2 (F-3): sc-376496 is recommended as a control antibody for monitoring of CLASP2 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 CLASP2 gene expression knockdown using RT-PCR Primer: CLASP2 (m)-PR: sc-142361-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

RESEARCH USE

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