

ZWINT siRNA (m): sc-63264

BACKGROUND

ZWINT (ZW10 interactor), also known as KNTC2AP or HZwint-1, is a 277 amino acid protein that is involved in kinetochore function. Localized to the cytoplasm during interphase and to kinetochores from late prophase to anaphase, ZWINT interacts with ZW10 (Zeste White 10) and functions to regulate the association between ZW10 and kinetochores. Additionally, ZWINT is part of a kinetochore complex composed of proteins such as MIS12 (MIND kinetochore complex component) and PMF-1 (polyamine-modulated factor 1) that work in concert to ensure proper kinetochore formation and spindle checkpoint activity. Defects in the gene encoding ZWINT are associated with the pathogenesis of Roberts syndrome, an autosomal recessive disorder characterized by growth retardation due to premature chromosome separation.

REFERENCES

1. Starr, D.A., et al. 2000. HZwint-1, a novel human kinetochore component that interacts with HZW10. *J. Cell Sci.* 113: 1939-1950.
2. Wang, H., et al. 2004. Human Zwint-1 specifies localization of Zeste White 10 to kinetochores and is essential for mitotic checkpoint signaling. *J. Biol. Chem.* 279: 54590-54598.
3. Obuse, C., et al. 2004. A conserved Mis12 centromere complex is linked to heterochromatic HP1 and outer kinetochore protein Zwint-1. *Nat. Cell Biol.* 6: 1135-1141.
4. Musio, A., et al. 2004. Recapitulation of the Roberts syndrome cellular phenotype by inhibition of INCENP, ZWINT-1 and ZW10 genes. *Gene* 331: 33-40.
5. Hirose, H., et al. 2004. Implication of ZW10 in membrane trafficking between the endoplasmic reticulum and Golgi. *EMBO J.* 23: 1267-1278.
6. Emanuele, M.J., et al. 2005. Measuring the stoichiometry and physical interactions between components elucidates the architecture of the vertebrate kinetochore. *Mol. Biol. Cell* 16: 4882-4892.
7. Kops, G.J., et al. 2005. ZW10 links mitotic checkpoint signaling to the structural kinetochore. *J. Cell Biol.* 169: 49-60.
8. Lin, Y.T., et al. 2006. Hec1 sequentially recruits Zwint-1 and ZW10 to kinetochores for faithful chromosome segregation and spindle checkpoint control. *Oncogene* 25: 6901-6914.

CHROMOSOMAL LOCATION

Genetic locus: Zwint (mouse) mapping to 10 B5.3.

PRODUCT

ZWINT 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 ZWINT shRNA Plasmid (m): sc-63264-SH and ZWINT shRNA (m) Lentiviral Particles: sc-63264-V as alternate gene silencing products.

For independent verification of ZWINT (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-63264A, sc-63264B and sc-63264C.

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

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

ZWINT (A-7): sc-271646 is recommended as a control antibody for monitoring of ZWINT 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 ZWINT gene expression knockdown using RT-PCR Primer: ZWINT (m)-PR: sc-63264-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.