

Zwilch siRNA (h): sc-63261

BACKGROUND

Zwilch is the human homolog of the *Drosophila* Zwilch protein. The *Drosophila* Zwilch forms a complex with both ROD (rough deal) and ZWINT (zeste-white 10, also designated Zw10) proteins. This complex is important for chromosome segregation because it recruits cytoplasmic Dynein to the kinetochore and plays a crucial role in the spindle checkpoint. The role of Zwilch in complex is thought to be evolutionarily conserved because the human homologs of Zwilch, ZWINT and ROD coimmunoprecipitate in the human HeLa cell line. The human Zwilch-ZWINT-ROD complex localizes to the kinetochores at pro-metaphase. Mutations were discovered in Zwilch-ZWINT-ROD during a screen for mutations in alleles encoding putative chromosome instability genes in cases of human colorectal cancer. These mutations may contribute in part to the chromosomal instability phenotype of colorectal tumor cells.

REFERENCES

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- Kops, G.J., Kim, Y., Weaver, B.A., Mao, Y., McLeod, I., Tagaya, M. and Cleveland, D.W. 2005. Zw10 links mitotic checkpoint signaling to the structural kinetochore. *J. Cell Biol.* 169: 49-60.
- Karess, R. 2005. ROD-Zw10-Zwilch: a key player in the spindle checkpoint. *Trends Cell Biol.* 15: 386-392.

CHROMOSOMAL LOCATION

Genetic locus: ZWILCH (human) mapping to 15q22.31.

PRODUCT

Zwilch 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Zwilch shRNA Plasmid (h): sc-63261-SH and Zwilch shRNA (h) Lentiviral Particles: sc-63261-V as alternate gene silencing products.

For independent verification of Zwilch (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-63261A, sc-63261B and sc-63261C.

PROTOCOLS

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

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

Zwilch (NB-C38): sc-135615 is recommended as a control antibody for monitoring of Zwilch 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 Zwilch gene expression knockdown using RT-PCR Primer: Zwilch (h)-PR: sc-63261-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.