

ROD siRNA (h): sc-62958

BACKGROUND

ROD (rough deal), also known as KNTC1 (kinetochore-associated protein 1), is the human homolog of the *Drosophila* Rod protein and is an essential component of the mitotic cell cycle checkpoint, functioning to assemble MAD1-MAD2 and Dynein-Dynactin complexes into kinetochores. Highly expressed in the testis, ROD exhibits a dynamic pattern of localization during the cell cycle; beginning at the nucleus and cytoplasm during interphase and translocating to kinetochores and spindle fibers during anaphase. ROD interacts with ZW10 and, through this interaction, is able to associate in a stable manner with the kinetochore. ROD and ZW10 are required for proper spindle assembly and help target microtubule motor cytoplasmic Dynein to kinetochores.

REFERENCES

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2. Scaërou, F., et al. 1999. The rough deal protein is a new kinetochore component required for accurate chromosome segregation in *Drosophila*. J. Cell Sci. 112: 3757-3768.
3. Chan, G.K., et al. 2000. Human Zw10 and ROD are mitotic checkpoint proteins that bind to kinetochores. Nat. Cell Biol. 2: 944-947.
4. Scaërou, F., et al. 2001. The ZW10 and Rough Deal checkpoint proteins function together in a large, evolutionarily conserved complex targeted to the kinetochore. J. Cell Sci. 114: 3103-3114.
5. Wojcik, E., et al. 2001. Kinetochore dynein: its dynamics and role in the transport of the Rough deal checkpoint protein. Nat. Cell Biol. 3: 1001-1007.
6. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607363. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
7. Williams, B.C., et al. 2003. Zwilch, a new component of the ZW10/ROD complex required for kinetochore functions. Mol. Biol. Cell 14: 1379-1391.
8. Basto, R., et al. 2004. *In vivo* dynamics of the rough deal checkpoint protein during *Drosophila* mitosis. Curr. Biol. 14: 56-61.

CHROMOSOMAL LOCATION

Genetic locus: KNTC1 (human) mapping to 12q24.31.

PRODUCT

ROD 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 ROD shRNA Plasmid (h): sc-62958-SH and ROD shRNA (h) Lentiviral Particles: sc-62958-V as alternate gene silencing products.

For independent verification of ROD (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-62958A, sc-62958B and sc-62958C.

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

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

ROD (43-K): sc-81853 is recommended as a control antibody for monitoring of ROD 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 ROD gene expression knockdown using RT-PCR Primer: ROD (h)-PR: sc-62958-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.