

BUBR1 siRNA (m): sc-37543

BACKGROUND

Human cells contain two related protein kinases, BUB1 and BUBR1, that appear to have evolved from a single ancestral BUB1 gene. Both kinases are concentrated near the surface of the kinetochore where they monitor kinetochore-microtubule interactions. BUB1 and BUBR1 bind to kinetochores and are postulated to be components of the mitotic checkpoint, which monitors kinetochore activities to determine if chromosomes have achieved alignment at the spindle equator. BUBR1 is essential for normal mitotic progression as it prevents cells from prematurely entering anaphase. BUB3 is a conserved component of the mitotic spindle assembly complex and is also involved with the essential spindle checkpoint pathway that operates during early embryogenesis.

REFERENCES

1. Donadelli, R., et al. 1998. Identification of a novel gene—SSK1—in human endothelial cells exposed to shear stress. *Biochem. Biophys. Res. Commun.* 246: 881-887.
2. Jablonski, S.A., et al. 1998. The hBUB1 and hBUBR1 kinases sequentially assemble onto kinetochores during prophase with hBUBR1 concentrating at the kinetochore plates in mitosis. *Chromosoma* 107: 386-396.
3. Chan, G.K., et al. 1999. Human BUBR1 is a mitotic checkpoint kinase that monitors CENP-E functions at kinetochores and binds the cyclosome/APC. *J. Cell Biol.* 146: 941-954.
4. Li, W., et al. 1999. BUBR1 phosphorylation is regulated during mitotic checkpoint activation. *Cell Growth Differ.* 10: 769-775.
5. Abrieu, A., et al. 2000. CENP-E as an essential component of the mitotic checkpoint *in vitro*. *Cell* 102: 817-826.
6. Kalitsis, P., et al. 2000. BUB3 gene disruption in mice reveals essential mitotic spindle checkpoint function during early embryogenesis. *Genes Dev.* 18: 2277-2282.

CHROMOSOMAL LOCATION

Genetic locus: Bub1b (mouse) mapping to 2 E5.

PRODUCT

BUBR1 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 BUBR1 shRNA Plasmid (m): sc-37543-SH and BUBR1 shRNA (m) Lentiviral Particles: sc-37543-V as alternate gene silencing products.

For independent verification of BUBR1 (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-37543A and sc-37543B.

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

BUBR1 siRNA (m) is recommended for the inhibition of BUBR1 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

BUBR1 (8G1): sc-47744 is recommended as a control antibody for monitoring of BUBR1 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 BUBR1 gene expression knockdown using RT-PCR Primer: BUBR1 (m)-PR: sc-37543-PR (20 μ l, 570 bp). 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.