SANTA CRUZ BIOTECHNOLOGY, INC.

BUB3 siRNA (m): sc-37541

BACKGROUND

BUB3 (budding uninhibited by benzimidazoles 3 homolog), also known as BUB3L or hBUB3, is a conserved component of the mitotic spindle assembly complex (MCC). It contains five WD repeat domains and forms cell cycle constitutive complexes with BUB1 and BUBR1. BUB3 is essential for the kinetochore localization of BUB1 and BUBR1. As a component of the MCC, BUB3 is involved in the essential spindle checkpoint pathway that operates during early embryogenesis. The spindle checkpoint pathway functions to postpone the initiation of anaphase until chromosomes are properly attached to the spindle. This acts to ensure accurate chromosome segregation. In addition, BUB3 plays a role in regulating the establishment of correct kinetochore-microtubule attachments. BUB3 is also thought to bind Tctex1L (or DYNLT3), a dynein light chain.

REFERENCES

1. Roberts, B.T., et al. 1994. The *Saccharomyces cerevisiae* checkpoint gene BUB1 encodes a novel protein kinase. Mol. Cell. Biol. 14: 8282-8291.
2. Guenette, S., et al. 1995. Suppression of a conditional mutation in α Tubulin by overexpression of two checkpoint genes. J. Cell Sci. 108: 1195-1204.
3. Farr, K.A., et al. 1998. BUB1p kinase activates the *Saccharomyces cerevisiae* spindle assembly checkpoint. Mol. Cell. Biol. 18: 2738-2747.
4. Martinez-Exposito, M.J., et al. 1999. Retention of the BUB3 checkpoint protein on lagging chromosomes. Proc. Natl. Acad. Sci. USA 96: 8493-8498.
5. Ru, H.Y., et al. 2002. hBUB1 defects in leukemia and lymphoma cells. Oncogene 21: 4673-4679.
6. Warren, C.D., et al. 2002. Distinct chromosome segregation roles for spindle checkpoint proteins. Mol. Biol. Cell 13: 3029-3041.
7. Lo, K.W., et al. 2007. The DYNLT3 light chain directly links cytoplasmic dynein to a spindle checkpoint protein, Bub3. J. Biol. Chem. 282: 11205-11212.
8. Braunstein, I., et al. 2007. Inhibitory factors associated with anaphase-promoting complex/cylosome in mitotic checkpoint. Proc. Natl. Acad. Sci. USA 104: 4870-4875.

CHROMOSOMAL LOCATION

Genetic locus: Bub3 (mouse) mapping to 7 F3.

PRODUCT

BUB3 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see BUB3 shRNA Plasmid (m): sc-37541-SH and BUB3 shRNA (m) Lentiviral Particles: sc-37541-V as alternate gene silencing products.

For independent verification of BUB3 (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-37541A, sc-37541B and sc-37541C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

BUB3 siRNA (m) is recommended for the inhibition of BUB3 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 µM in 66 µl. 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

BUB3 (E-7): sc-376506 is recommended as a control antibody for monitoring of BUB3 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 BUB3 gene expression knockdown using RT-PCR Primer: BUB3 (m)-PR: sc-37541-PR (20 µl). 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.