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SANTA CRUZ BIOTECHNOLOGY, INC.

UBC13 siRNA (h): sc-43551

BACKGROUND

Ubiquitination is an important mechanism through which three classes of enzymes act in concert to target short-lived or abnormal proteins for destruction. The three classes of enzymes involved in ubiquitination are the ubiquitin-activating enzymes (E1s), the ubiquitin-conjugating enzymes (E2s) and the ubiquitin-protein ligases (E3s). UBC13, also known as UBE2N or BLU, is a 152 amino acid member of the E2 ubiquitin-conjugating enzyme family. Existing as a heterodimer with Mms2 (also known as UBE2V2), UBC13 catalyzes the ATP-dependent synthesis of non-canonical polyubiquitin chains, a process that does not lead to proteasomal degradation. Additionally, UBC13 mediates the transcription of several target genes and is thought to play a role in cell cycle progression, cellular differentiation and DNA repair mechanisms that ensure cell survival after DNA damage.

REFERENCES

1. Yamaguchi, T., et al. 1996. Cloning and expression of cDNA encoding a human ubiquitin-conjugating enzyme similar to the *Drosophila* bendless gene product. J. Biochem. 120: 494-497.
2. Hoege, C., et al. 2002. Rad6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature 419: 135-141.
3. Andersen, P.L., et al. 2005. Distinct regulation of UBC13 functions by the two ubiquitin-conjugating enzyme variants Mms2 and Uev1A. J. Cell Biol. 170: 745-755.
4. Plans, V., et al. 2006. The RING finger protein RNF8 recruits UBC13 for lysine 63-based self polyubiquitylation. J. Cell. Biochem. 97: 572-582.

CHROMOSOMAL LOCATION

Genetic locus: UBE2N (human) mapping to 12q22.

PRODUCT

UBC13 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UBC13 shRNA Plasmid (h): sc-43551-SH and UBC13 shRNA (h) Lentiviral Particles: sc-43551-V as alternate gene silencing products.

For independent verification of UBC13 (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-43551A, sc-43551B and sc-43551C.

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

UBC13 siRNA (h) is recommended for the inhibition of UBC13 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 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

UBC13 (F-10): sc-376470 is recommended as a control antibody for monitoring of UBC13 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 UBC13 gene expression knockdown using RT-PCR Primer: UBC13 (h)-PR: sc-43551-PR (20 µl, 395 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Wang, S.C., et al. 2006. Tyrosine phosphorylation controls PCNA function through protein stability. Nat. Cell Biol. 8: 1359-1368.
2. Tripathi, E. and Smith, S. 2017. Cell cycle-regulated ubiquitination of tankyrase 1 by RNF8 and ABRO1/BRCC36 controls the timing of sister telomere resolution. EMBO J. 36: 503-519.

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.