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UBC13 shRNA (h) Lentiviral Particles: sc-43551-V

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

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2. Hoegge, 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.
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CHROMOSOMAL LOCATION

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

PRODUCT

UBC13 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see UBC13 siRNA (h): sc-43551 and UBC13 shRNA Plasmid (h): sc-43551-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

UBC13 shRNA (h) Lentiviral Particles is recommended for the inhibition of UBC13 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 μ l, 395 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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