

# UBE2B siRNA (h): sc-106915

## BACKGROUND

The ubiquitin (Ub) pathway involves three sequential enzymatic steps that facilitate the conjugation of Ub and Ub-like molecules to specific protein substrates. The first step requires the ATP-dependent activation of the Ub C-terminus and the assembly of multi-Ub chains by the Ub-activating enzyme known as the E1 component. The Ub chain is then conjugated to the Ub-conjugating enzyme (E2) to generate an intermediate Ub-E2 complex. The Ub-ligase (E3) then catalyzes the transfer of Ub from E2 to the appropriate protein substrate. UBE2B (ubiquitin-conjugating enzyme E2B), also designated HR6B, UBC2, HHR6B, RAD6B or E2-17kDa, is a 152 amino acid protein that is expressed in the plasma membrane and in nuclei. Belonging to the ubiquitin-conjugating enzyme family, UBE2B is required for post-replication repair of UV-damaged DNA and associates to the E3 ligase Rad18 to form the UBE2B-Rad18 ubiquitin ligase complex, which is involved in mono-ubiquitination of DNA-associated PCNA.

## REFERENCES

1. Aguilar-Mahecha, A., et al. 2001. Expression of stress response genes in germ cells during spermatogenesis. *Biol. Reprod.* 65: 119-127.
2. Sasaki, H., et al. 2004. Decreased HRad6B expression in lung cancer. *Acta Oncol.* 43: 585-589.
3. Roest, H.P., et al. 2004. The ubiquitin-conjugating DNA repair enzyme HR6A is a maternal factor essential for early embryonic development in mice. *Mol. Cell. Biol.* 24: 5485-5495.
4. Shekhar, M.P., et al. 2006. Essential role of T cell factor/ $\beta$ -catenin in regulation of Rad6B: a potential mechanism for Rad6B overexpression in breast cancer cells. *Mol. Cancer Res.* 4: 729-745.

## CHROMOSOMAL LOCATION

Genetic locus: UBE2B (human) mapping to 5q31.1.

## PRODUCT

UBE2B 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UBE2B shRNA Plasmid (h): sc-106915-SH and UBE2B shRNA (h) Lentiviral Particles: sc-106915-V as alternate gene silencing products.

For independent verification of UBE2B (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-106915A, sc-106915B and sc-106915C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

UBE2B siRNA (h) is recommended for the inhibition of UBE2B 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  $\mu$ M in 66  $\mu$ 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

UBE2A/B (G-9): sc-365507 is recommended as a control antibody for monitoring of UBE2B 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor UBE2B gene expression knockdown using RT-PCR Primer: UBE2B (h)-PR: sc-106915-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Chen, S., et al. 2015. RAD6 promotes homologous recombination repair by activating the autophagy-mediated degradation of heterochromatin protein HP1. *Mol. Cell. Biol.* 35: 406-416.
2. Wang, C., et al. 2015. A conserved RAD6-MDM2 ubiquitin ligase machinery targets histone chaperone ASF1A in tumorigenesis. *Oncotarget* 6: 29599-29613.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.