

# UBC9 siRNA (m): sc-36774

## BACKGROUND

UBC9 is a component of the ubiquitin-mediated proteolytic pathway, which targets proteins for degradation by the 26S Proteasome, mediates endocytosis and directs protein subcellular localization. Ub and Ub-like molecules are systematically transferred from E2 conjugating enzymes to the targeted substrate by way of an E3 ubiquitin ligase. UBC9 functions as an E2 ubiquitin conjugating enzyme that preferentially associates with the ubiquitin homolog designated SUMO-1 or sentrin, a component of the sentrinization complex. Characteristic of the E2 family members, UBC9 contains a conserved cysteine residue that is required for the thio ester formation between Ub-like proteins and the E2 member, and it shares a conserved UBC domain. Substrates for UBC9 include transcription factors E12 and E47 and mitotic regulators RanBP2 and RanGAP1, which indicates that UBC9 may regulate various cellular processes including cell cycle progression and differentiation.

## REFERENCES

1. Jentsch, S. 1992. The ubiquitin-conjugation system. *Annu. Rev. Genet.* 26: 179-207.
2. Wang, Z.Y., et al. 1996. Molecular cloning of the cDNA and chromosome localization of the gene for human ubiquitin-conjugating enzyme 9. *J. Biol. Chem.* 271: 24811-24816.
3. Hochstrasser, M. 1996. Protein degradation or regulation: Ub the judge. *Cell* 84: 813-815.
4. Gong, L., et al. 1997. Preferential interaction of sentrin with a ubiquitin-conjugating enzyme, UBC9. *J. Biol. Chem.* 272: 28198-28201.

## CHROMOSOMAL LOCATION

Genetic locus: Ube2i (mouse) mapping to 17 A3.3.

## PRODUCT

UBC9 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UBC9 shRNA Plasmid (m): sc-36774-SH and UBC9 shRNA (m) Lentiviral Particles: sc-36774-V as alternate gene silencing products.

For independent verification of UBC9 (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-36774A, sc-36774B and sc-36774C.

## 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

UBC9 siRNA (m) is recommended for the inhibition of UBC9 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  $\mu$ 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

UBC9 (C-12): sc-271057 is recommended as a control antibody for monitoring of UBC9 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 UBC9 gene expression knockdown using RT-PCR Primer: UBC9 (m)-PR: sc-36774-PR (20  $\mu$ l, 503 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Yuan, Y.F., et al. 2014. SUMO-1 plays crucial roles for spindle organization, chromosome congression, and chromosome segregation during mouse oocyte meiotic maturation. *Mol. Reprod. Dev.* 81: 712-724.
2. Tomasi, M.L., et al. 2014. Molecular mechanisms of lipopolysaccharide-mediated inhibition of glutathione synthesis in mice. *Free Radic. Biol. Med.* 68: 148-158.
3. Digiacoio, V., et al. 2015. The transition of the 37-kDa Laminin receptor (RPSA) to higher molecular weight species: sumoylation or artifact? *Cell. Mol. Biol. Lett.* 20: 571-585.

## RESEARCH USE

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