

# RNF8 siRNA (m): sc-61485

## BACKGROUND

The RING finger motif is a specialized DNA-binding zinc finger domain found in many transcriptional regulatory proteins. The RING finger protein (RNF) family includes any protein containing the signature RING finger motif. RNF8 is a ubiquitously expressed nuclear RING finger protein that acts as an E3 ubiquitin-protein ligase. It is required for the ubiquitination of some nuclear proteins and promotes their subsequent degradation. The heterodimeric ubiquitin-conjugating enzyme UBC13 interacts with RNF8, and they co-localize in the nucleus. RNF8 may regulate mediation of UBC13 polyubiquitylation by elongating the ubiquitin chains. RNF8 also binds to Retinoid X receptor  $\alpha$  (RXR $\alpha$ ), a member of the steroid hormone receptor superfamily. It increases RXR $\alpha$ -mediated transactivation of the RXR $\alpha$ -responsive element (RXRE) promoter in a dose-dependent manner, suggesting that RNF8 is a regulator of RXR $\alpha$ -mediated transcriptional activity.

## REFERENCES

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- Ito, K., et al. 2001. N-terminally extended human ubiquitin-conjugating enzymes (E2s) mediate the ubiquitination of RING finger proteins ARA54 and RNF8. *Eur. J. Biochem.* 268: 2725-2732.
- Pringa, E., et al. 2001. Interaction of the RING finger-related U-box motif of a nuclear dot protein with ubiquitin-conjugating enzymes. *J. Biol. Chem.* 276: 19617-19623.
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- Hirvonen-Santti, S.J., et al. 2004. Small nuclear RING finger protein expression during gonad development: regulation by gonadotropins and estrogen in the postnatal ovary. *Endocrinology* 145: 2433-2444.
- Takano, Y., et al. 2004. The RING finger protein, RNF8, interacts with retinoid X receptor  $\alpha$  and enhances its transcription-stimulating activity. *J. Biol. Chem.* 279: 18926-18934.

## CHROMOSOMAL LOCATION

Genetic locus: Rnf8 (mouse) mapping to 17 A3.3.

## PRODUCT

RNF8 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 RNF8 shRNA Plasmid (m): sc-61485-SH and RNF8 shRNA (m) Lentiviral Particles: sc-61485-V as alternate gene silencing products.

For independent verification of RNF8 (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-61485A, sc-61485B and sc-61485C.

## 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

RNF8 siRNA (m) is recommended for the inhibition of RNF8 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

RNF8 (B-2): sc-271462 is recommended as a control antibody for monitoring of RNF8 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 RNF8 gene expression knockdown using RT-PCR Primer: RNF8 (m)-PR: sc-61485-PR (20  $\mu$ l). 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](http://www.scbt.com) for detailed protocols and support products.