

# RNF25 siRNA (m): sc-153044

## 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). RNF25 (RING finger protein 25), also known as A07, is a 459 amino acid protein that contains one RWD domain and one RING-type zinc finger. Via its RING-type zinc finger (a common domain that contains a conserved cysteine-rich region and is found in a number of viral and eukaryotic proteins), RNF25 is thought to exhibit E2-dependent E3 ubiquitin-protein ligase activity. Specifically, the RING finger of RNF25 interacts with an E2 ligase and, through this interaction, facilitates the ubiquitination of target proteins. RNF25 may also regulate the transcriptional activity of NF $\kappa$ B p65, a protein that plays important roles in cell survival, inflammation response, host defense and proliferation.

## REFERENCES

1. Borden, K.L. and Freemont, P.S. 1996. The RING finger domain: a recent example of a sequence-structure family. *Curr. Opin. Struct. Biol.* 6: 395-401.
2. Lorick, K.L., Jensen, J.P., Fang, S., Ong, A.M., Hatakeyama, S. and Weissman, A.M. 1999. RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc. Natl. Acad. Sci. USA* 96: 11364-11369.
3. Asamitsu, K., Tetsuka, T., Kanazawa, S. and Okamoto, T. 2003. RING finger protein A07 supports NF $\kappa$ B-mediated transcription by interacting with the transactivation domain of the p65 subunit. *J. Biol. Chem.* 278: 26879-26887.
4. Colland, F., Jacq, X., Trouplin, V., Mougin, C., Groizeleau, C., Hamburger, A., Meil, A., Wojcik, J., Legrain, P. and Gauthier, J.M. 2004. Functional proteomics mapping of a human signaling pathway. *Genome Res.* 14: 1324-1332.
5. Penengo, L., Mapelli, M., Murachelli, A.G., Confalonieri, S., Magri, L., Musacchio, A., Di Fiore, P.P., Polo, S. and Schneider, T.R. 2006. Crystal structure of the ubiquitin binding domains of rabex-5 reveals two modes of interaction with ubiquitin. *Cell* 124: 1183-1195.

## CHROMOSOMAL LOCATION

Genetic locus: Rnf25 (mouse) mapping to 1 C3.

## PRODUCT

RNF25 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 RNF25 shRNA Plasmid (m): sc-153044-SH and RNF25 shRNA (m) Lentiviral Particles: sc-153044-V as alternate gene silencing products.

For independent verification of RNF25 (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-153044A, sc-153044B and sc-153044C.

## 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

RNF25 siRNA (m) is recommended for the inhibition of RNF25 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

RNF25 (E-10): sc-398749 is recommended as a control antibody for monitoring of RNF25 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>TM</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 RNF25 gene expression knockdown using RT-PCR Primer: RNF25 (m)-PR: sc-153044-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.