

# RNF20 siRNA (h): sc-92753

## 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). RNF20 (ring finger protein 20), also known as BRE1, BRE1A or hBRE1, is a 975 amino acid nuclear protein that belongs to the BRE1 family. As a component of the RNF20/40 complex, RNF20 functions as an E3 ubiquitin-protein ligase that regulates the monoubiquitination and subsequent degradation of select residues on target proteins, such as Histone H2B. RNF20 is required for transcriptional activation of Hox genes and is most likely recruited by p53 to the MDM2 promoter, thereby acting as a transcriptional co-activator. RNF20 contains one zinc finger domain and exists as a homodimer.

## REFERENCES

1. Zhu, B., et al. 2005. Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. *Mol. Cell* 20: 601-611.
2. Kim, J., et al. 2005. The human homolog of yeast BRE1 functions as a transcriptional coactivator through direct activator interactions. *Mol. Cell* 20: 759-770.
3. Shema, E., et al. 2008. The histone H2B-specific ubiquitin ligase RNF20/hBRE1 acts as a putative tumor suppressor through selective regulation of gene expression. *Genes Dev.* 22: 2664-2676.
4. Buro, L.J., et al. 2010. Menin and RNF20 recruitment is associated with dynamic histone modifications that regulate signal transducer and activator of transcription 1 (STAT1)-activated transcription of the interferon regulatory factor 1 gene (IRF1). *Epigenetics Chromatin* 3: 16.
5. Gao, Z., et al. 2011. Resveratrol induces cellular senescence with attenuated mono-ubiquitination of histone H2B in glioma cells. *Biochem. Biophys. Res. Commun.* 407: 271-276.
6. Taniue, K., et al. 2011. A member of the ETS family, EHF, and the ATPase RUVBL1 inhibit p53-mediated apoptosis. *EMBO Rep.* 12: 682-689.
7. Zhang, F., et al. 2011. WAC, a functional partner of RNF20/40, regulates histone H2B ubiquitination and gene transcription. *Mol. Cell* 41: 384-397.

## CHROMOSOMAL LOCATION

Genetic locus: RNF20 (human) mapping to 9q31.1.

## PRODUCT

RNF20 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 RNF20 shRNA Plasmid (h): sc-92753-SH and RNF20 shRNA (h) Lentiviral Particles: sc-92753-V as alternate gene silencing products.

For independent verification of RNF20 (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-92753A, sc-92753B and sc-92753C.

## 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

RNF20 siRNA (h) is recommended for the inhibition of RNF20 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

RNF20 (1594CT552.128.36): sc-517358 is recommended as a control antibody for monitoring of RNF20 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 RNF20 gene expression knockdown using RT-PCR Primer: RNF20 (h)-PR: sc-92753-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.