

XRN2 siRNA (m): sc-61814

BACKGROUND

Degradation of mRNA is a critical aspect of gene expression that occurs via the exoribonuclease. Exoribonuclease 2 (XRN2) is the human homologue of the *Saccharomyces cerevisiae* RAT1, which functions as a nuclear 5' to 3' exoribonuclease and is essential for mRNA turnover and cell viability. XRN2 also processes rRNAs and small nucleolar RNAs (snoRNAs) in the nucleus. XRN2 moves along with RNA polymerase II and gains access to the nascent RNA transcript after the endonucleolytic cleavage at the poly(A) site or at a second cotranscriptional cleavage site (CoTC). CoTC is an autocatalytic RNA structure that undergoes rapid self-cleavage and acts as a precursor to termination by presenting a free RNA 5' end to be recognized by XRN2. XRN2 then travels in a 5'-3' direction like a guided torpedo and facilitates the dissociation of the RNA polymerase elongation complex.

REFERENCES

- Shobuie, T., et al. 1995. Characterization of cDNA encoding mouse homologue of fission yeast dhp1+ gene: structural and functional conservation. *Nucleic Acids Res.* 23: 357-361.
- Zhang, M., et al. 1999. Cloning and mapping of the XRN2 gene to human chromosome 20p11.1-p11.2. *Genomics* 59: 252-254.
- Kastenmayer, J.P., et al. 2000. Novel features of the XRN-family in *Arabidopsis*: evidence that AtXRN4, one of several orthologs of nuclear Xrn2p/Rat1p, functions in the cytoplasm. *Proc. Natl. Acad. Sci. USA* 97: 13985-13990.
- Johnson, A.W. 2001. Rat1p nuclease. *Methods Enzymol.* 342: 260-268.

CHROMOSOMAL LOCATION

Genetic locus: Xrn2 (mouse) mapping to 2 G2.

PRODUCT

XRN2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see XRN2 shRNA Plasmid (m): sc-61814-SH and XRN2 shRNA (m) Lentiviral Particles: sc-61814-V as alternate gene silencing products.

For independent verification of XRN2 (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-61814A, sc-61814B and sc-61814C.

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

APPLICATIONS

XRN2 siRNA (m) is recommended for the inhibition of XRN2 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 μ M in 66 μ 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

XRN2 (H-3): sc-365258 is recommended as a control antibody for monitoring of XRN2 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 κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor XRN2 gene expression knockdown using RT-PCR Primer: XRN2 (m)-PR: sc-61814-PR (20 μ 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 for detailed protocols and support products.