

PNGase siRNA (m): sc-152352

BACKGROUND

PNGase, also known as NGLY1 (N-glycanase 1) or PNG1, is a 654 amino acid protein that localizes to the cytoplasm and contains one PAW domain and one PUB domain. Using zinc as a cofactor, PNGase deglycosylates the denatured form of cytoplasmic N-linked glycoproteins, specifically cleaving the β -aspartyl-glucosamine residue in the target protein and assisting in proteasome-mediated degradation. PNGase is also capable of recognizing and deglycosylating misfolded proteins in the endoplasmic reticulum (ER), thereby playing a role in the elimination of misfolded glycoproteins. PNGase exists as four alternatively spliced isoforms and is functionally inhibited by Z-VAD-fmk, a caspase inhibitor that binds to PNGase and inhibits its enzymatic activity.

REFERENCES

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CHROMOSOMAL LOCATION

Genetic locus: Ngly1 (mouse) mapping to 14 A2.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

PRODUCT

PNGase 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 PNGase shRNA Plasmid (m): sc-152352-SH and PNGase shRNA (m) Lentiviral Particles: sc-152352-V as alternate gene silencing products.

For independent verification of PNGase (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-152352A, sc-152352B and sc-152352C.

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

PNGase siRNA (m) is recommended for the inhibition of PNGase 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PNGase gene expression knockdown using RT-PCR Primer: PNGase (m)-PR: sc-152352-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.