

PARG siRNA (m): sc-152026

BACKGROUND

The synthesis and rapid turnover of ADP-ribose polymers is an immediate cellular response to DNA damage. Poly(ADP-ribose) is a reversible covalent-modifier to chromosomal proteins and is synthesized by poly (ADP-ribose) polymerase (PARP-1) and other related enzymes. Poly(ADP-ribose) glycohydrolase (PARG) is the enzyme responsible for polymer turnover. Under normal growth conditions, PARG localizes to the cytoplasm. PARG is an enzymatically active protein that is cleaved to multiple fragments. PARG is cleaved during etoposide-, staurosporine-, and Fas-induced apoptosis in human cells by caspases, and generates two C-terminal fragments, which still contain the active site of the enzyme required to hydrolyze poly (ADP-ribose). Under normal growth, PARG is expressed only as a doublet by SDS-PAGE. The gene encoding PARG maps to human chromosome 10q11.23.

REFERENCES

1. Lin, W., et al. 1997. Isolation and characterization of the cDNA encoding bovine poly(ADP-ribose) glycohydrolase. *J. Biol. Chem.* 272: 11895-11901.
2. Winstall, E., et al. 1999. Poly(ADP-ribose) glycohydrolase is present and active in mammalian cells as a 110-kDa protein. *Exp. Cell Res.* 246: 395-398.
3. D'Amours, D., et al. 1999. Poly(ADP-ribosylation) reactions in the regulation of nuclear functions. *Biochem. J.* 342: 249-268.
4. Ame, J.C., et al. 1999. Assignment of poly(ADP-ribose) glycohydrolase gene (PARG) to human chromosome 10q11.23 and mouse chromosome 14B by *in situ* hybridization. *Cytogenet. Cell Genet.* 85: 269-270.
5. Affar, E.B., et al. 2001. Caspase-3-mediated processing of poly(ADP-ribose) glycohydrolase during apoptosis. *J. Biol. Chem.* 276: 2935-2942.

CHROMOSOMAL LOCATION

Genetic locus: Parg (mouse) mapping to 14 B.

PRODUCT

PARG 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 PARG shRNA Plasmid (m): sc-152026-SH and PARG shRNA (m) Lentiviral Particles: sc-152026-V as alternate gene silencing products.

For independent verification of PARG (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-152026A, sc-152026B and sc-152026C.

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.

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

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

PARG (H-1): sc-398563 is recommended as a control antibody for monitoring of PARG 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 PARG gene expression knockdown using RT-PCR Primer: PARG (m)-PR: sc-152026-PR (20 μ l, 537 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.