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PUMA siRNA (r): sc-270040

BACKGROUND

PUMA (Bcl-2 binding component 3, JFY1, PUMA/JFY1) is a mitochondrial pro-apoptotic Bcl-2 homology domain (BH3)-only protein that induces rapid apoptosis through a Bax- and mitochondria-dependent pathway. The PUMA gene encodes four proteins originating from different splice variants of the same transcript: PUMA α , β , γ and δ . Both PUMA α and PUMA β contain a BH3 domain, while PUMA γ and PUMA δ lack this domain. The BH3 domain is essential for binding of PUMA α and PUMA β to Bcl-2 or Bcl-x_L. PUMA is an initiator of γ -radiation apoptosis and glucocorticoid-induced apoptosis in lymphoid cells *in vivo*. Bcl-2 family members generally regulate apoptosis and transmit death signals to mitochondria. Members of this family include both pro- and anti-apoptotic proteins that share homologous sequences known as Bcl-2 homology domains (BH1-4). The BH3 proteins, BID, NOXA, PUMA, NBK, Bim and Bad, are all pro-apoptotic and share sequence homology within the amphipathic α -helical BH3 region.

REFERENCES

- Han, J., et al. 2001. Expression of BBC3, a pro-apoptotic BH3-only gene, is regulated by diverse cell death and survival signals. *Proc. Natl. Acad. Sci. USA* 98: 11318-11323.
- Yu, J., et al. 2001. PUMA induces the rapid apoptosis of colorectal cancer cells. *Mol. Cell* 7: 673-682.
- Nakano, K., et al. 2001. PUMA, a novel pro-apoptotic gene, is induced by p53. *Mol. Cell* 7: 683-694.
- Bouillet, P., et al. 2002. BH3-only proteins—evolutionarily conserved pro-apoptotic Bcl-2 family members essential for initiating programmed cell death. *J. Cell Sci.* 115: 1567-1574.

CHROMOSOMAL LOCATION

Genetic locus: Bbc3 (rat) mapping to 1q21.

PRODUCT

PUMA siRNA (r) is a pool of 2 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 PUMA shRNA Plasmid (r): sc-270040-SH and PUMA shRNA (r) Lentiviral Particles: sc-270040-V as alternate gene silencing products.

For independent verification of PUMA (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270040A and sc-270040B.

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

PUMA siRNA (r) is recommended for the inhibition of PUMA expression in rat 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

PUMA α (B-6): sc-377015 is recommended as a control antibody for monitoring of PUMA 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 PUMA gene expression knockdown using RT-PCR Primer: PUMA (r)-PR: sc-270040-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Yan, J., et al. 2011. Blood-brain barrier disruption following subarachnoid hemorrhage may be facilitated through PUMA induction of endothelial cell apoptosis from the endoplasmic reticulum. *Exp. Neurol.* 230: 240-247.
- Li, D., et al. 2013. Doxorubicin-induced apoptosis in H9c2 cardiomyocytes by NF κ B dependent PUMA upregulation. *Eur. Rev. Med. Pharmacol. Sci.* 17: 2323-2329.

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.