SANTA CRUZ BIOTECHNOLOGY, INC.

RIP3 siRNA (h): sc-61482

BACKGROUND

The death domain is a cytoplasmic domain of approximately 80 amino acids that is necessary for the transduction of apoptotic signals and is present in the apoptosis-mediating receptors TNF-R1 and FAS. Other death domain-containing, but otherwise structurally unrelated proteins have been identified on the basis of their ability to associate with the cytoplasmic domains of TNF-R1 or FAS. One of these proteins, the receptor-interacting protein 3 (RIP3), contains an N-terminal kinase domain and shares extensive homology with RIP and RIP2. However, RIP3 contains a unique C-terminal death domain, which promotes apoptosis. RIP3 can be expressed as two splice variants, RIP3β and RIP3γ, which contain a truncated N-terminal kinase domain and a distinct and shorter C-terminus. Subsequently, expression of these splice variants downregulates RIP3-mediated apoptosis.

CHROMOSOMAL LOCATION

Genetic locus: RIPK3 (human) mapping to 14q12.

PRODUCT

RIP3 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RIP3 shRNA Plasmid (h): sc-61482-SH and RIP3 shRNA (h) Lentiviral Particles: sc-61482-V as alternate gene silencing products.

For independent verification of RIP3 (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-61482A, sc-61482B and sc-61482C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

RIP3 siRNA (h) is recommended for the inhibition of RIP3 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 µM in 66 µl. 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

RIP3 (B-2): sc-374639 is recommended as a control antibody for monitoring of RIP3 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 RIP3 gene expression knockdown using RT-PCR Primer: RIP3 (h)-PR: sc-61482-PR (20 µl, 598 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Zhang, N., et al. 2011. PARP and RIP 1 are required for autophagy induced by 11'-deoxyverticillin A, which precedes caspase-dependent apoptosis. Autophagy 7: 598-612.
2. He, W., et al. 2014. A JNK-mediated autophagy pathway that triggers c-IAP degradation and necroptosis for anticancer chemotherapy. Oncogene 33: 3004-3013.
3. Zhang, M., et al. 2015. The roles of Ros and caspases in TRAIL-induced apoptosis and necroptosis in human pancreatic cancer cells. PLoS ONE 10: e0127386.
4. Miki, Y., et al. 2015. Photodynamic therapy using talaporfin sodium induces concentration-dependent programmed necroptosis in human glioblastoma T98G cells. Lasers Med. Sci. 30: 1739-1745.
5. Zhou, H., et al. 2018. Ripk3 regulates cardiac microvascular reperfusion injury: The role of IP3R-dependent calcium overload, XO-mediated oxidative stress and F-action/filopodia-based cellular migration. Cell. Signal. 45: 12-22.
6. Yan, S., et al. 2021. Necroptosis pathway blockage attenuates PFKFB3 inhibitor-induced cell viability loss and genome instability in colorectal cancer cells. Am. J. Cancer Res. 11: 2062-2080.

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.