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# RIP shRNA (h2) Lentiviral Particles: sc-44326-V

## BACKGROUND

In contrast to growth factors which promote cell proliferation, FAS ligand (FAS-L) and the tumor necrosis factors (TNFs) rapidly induce apoptosis. Cellular response to FAS-L and TNF is mediated by structurally related receptors containing a conserved "death domain" and belonging to the TNF receptor superfamily. TRADD, FADD and RIP are FAS/TNF-R1 interacting proteins that contain a death domain homologous region (DDH). TRADD (TNF-R1-associated death domain) and FADD (FAS-associated death domain) associate with the death domains of both FAS and TNF-R1 via their DDH regions. Overexpression of TRADD leads to NF $\kappa$ B activation and apoptosis in the absence of TNF. Overexpression of FADD causes apoptosis, which can be blocked by the cow pox protein CrmA, suggesting that FADD lies upstream of ICE and possibly other serine proteases. The receptor interacting protein, RIP, associates with FAS exclusively via its DDH and this association is abrogated in *lpr* mutants. Unlike TRADD and FADD, RIP contains a putative amino terminal kinase domain.

## REFERENCES

1. Smith, C.A., et al. 1994. The TNF receptor superfamily of cellular and viral proteins: activation, costimulation and death. *Cell* 76: 959-962.
2. Nagata, S., et al. 1995. The Fas death factor. *Science* 267: 1449-1456.
3. Sato, T., et al. 1995. FAP-1: a protein tyrosine phosphatase that associates with Fas. *Science* 268: 411-414.
4. Cleveland, J.L. and Ihle JN. 1995. Contenders in FAS-L/TNF death signaling. *Cell* 81: 479-482.
5. Hsu, H., et al. 1995. The TNF receptor 1-associated protein TRADD signals cell death and NF $\kappa$ B activation. *Cell* 81: 495-504.
6. Chinnaiyan, A.M., et al. 1995. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 81: 505-512.

## CHROMOSOMAL LOCATION

Genetic locus: RIPK1 (human) mapping to 6p25.2.

## PRODUCT

RIP shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see RIP siRNA (h2): sc-44326 and RIP shRNA Plasmid (h2): sc-44326-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

RIP shRNA (h2) Lentiviral Particles is recommended for the inhibition of RIP expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

RIP (C-12): sc-133102 is recommended as a control antibody for monitoring of RIP 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor RIP gene expression knockdown using RT-PCR Primer: RIP (h2)-PR: sc-44326-PR (20  $\mu$ l, 514 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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