

RIG-I siRNA (r): sc-270587

BACKGROUND

The innate immune system senses viral infection by recognizing many different viral components and triggering specific antiviral responses. Intracellular double-stranded RNA (dsRNA) is a major sign of replication for many viruses. Retinoic acid inducible gene I (RIG-I) is a 925 amino acid, interferon-inducible cellular DExD/H box RNA helicase that activates type I interferon (IFN), an important effector of the innate immune system that is sensitive to these dsRNA viruses. dsRNA is normally present in very low quantities in cells, so when a virus is present, the elevated levels of dsRNA act as a sign telling RIG-I to activate the production of IFN. RIG-I does this by using its helicase domain to bind to viral dsRNA, thus transmitting the activation signal for IFN through I κ B kinase-related kinases and inducing IFN expression. RIG-I is expressed in the cytoplasm of fibroblasts and conventional dendritic cells and can distinguish between many different RNA viruses.

REFERENCES

1. Sumpter, R., et al. 2005. Regulating intracellular antiviral defense and permissiveness to Hepatitis C virus RNA replication through a cellular RNA helicase, RIG-I. *J. Virol.* 79: 2689-2699.
2. Breiman, A., et al. 2005. Inhibition of RIG-I-dependent signaling to the interferon pathway during hepatitis C virus expression and restoration of signaling by IKK ϵ . *J. Virol.* 79: 3969-3978.
3. Fensterl, V., et al. 2005. Hepatitis A virus suppresses RIG-I-mediated IRF-3 activation to block induction of β interferon. *J. Virol.* 79: 10968-10977.
4. Kato, H., et al. 2005. Cell type-specific involvement of RIG-I in antiviral response. *Immunity* 23: 19-28.

CHROMOSOMAL LOCATION

Genetic locus: Ddx58 (rat) mapping to 5q22.

PRODUCT

RIG-I siRNA (r) 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 RIG-I shRNA Plasmid (r): sc-270587-SH and RIG-I shRNA (r) Lentiviral Particles: sc-270587-V as alternate gene silencing products.

For independent verification of RIG-I (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-270587A, sc-270587B and sc-270587C.

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

RIG-I siRNA (r) is recommended for the inhibition of RIG-I 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

RIG-I (D-12): sc-376845 is recommended as a control antibody for monitoring of RIG-I 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 RIG-I gene expression knockdown using RT-PCR Primer: RIG-I (r)-PR: sc-270587-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

1. Zhang, C., et al. 2021. The RIG-I signal pathway mediated *Panax notoginseng* saponin anti-inflammatory effect in ischemia stroke. *Evid. Based Complement. Alternat. Med.* 2021: 8878428.

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.