

Int-6 siRNA (r): sc-270194

BACKGROUND

Int-6, also designated eIF3e, eIF3-p46, eIF3-p48 and eukaryotic translation initiation factor 3, subunit 6, regulates translation and protein degradation through binding with three complexes: the eukaryotic translation initiation factor 3 (eIF3), the proteasome regulatory lid and the constitutive photomorphogenesis 9 signalosome. eIF3 is a complex that mediates assembly of 40S ribosomal subunits on mRNA bearing either a 5'-cap or an internal ribosome entry site (IRES). The Int-6 gene is a site of mouse mammary tumor virus (MMTV) integration in murine tumors. Reducing Int-6 expression by RNA interference in HeLa cells alters mitosis progression through defects in spindle formation, chromosome segregation and cytokinesis. These aberrations appear to correlate with an inhibition of cyclin B-Cdk1 kinase activity, due to a protracted inhibitory phosphorylated state of Cdk1.

REFERENCES

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- Rasmussen, S.B., et al. 2001. Evidence for the transforming activity of a truncated Int-6 gene, *in vitro*. *Oncogene* 20: 5291-5301.
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- von Arnim, A.G., et al. 2003. Protein homeostasis: a degrading role for Int-6/eIF3e. *Curr. Biol.* 13: R323-R325.
- Watkins, S.J., et al. 2004. Cell cycle-related variation in subcellular localization of eIF3e/Int-6 in human fibroblasts. *Cell Prolif.* 37: 149-160.

CHROMOSOMAL LOCATION

Genetic locus: Eif3el1 (rat) mapping to 7.

PRODUCT

Int-6 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 Int-6 shRNA Plasmid (r): sc-270194-SH and Int-6 shRNA (r) Lentiviral Particles: sc-270194-V as alternate gene silencing products.

For independent verification of Int-6 (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-270194A, sc-270194B and sc-270194C.

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

Int-6 siRNA (r) is recommended for the inhibition of Int-6 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

Int-6 (A-11): sc-133251 is recommended as a control antibody for monitoring of Int-6 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 Int-6 gene expression knockdown using RT-PCR Primer: Int-6 (r)-PR: sc-270194-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.