Int-6 siRNA (m): sc-40562



The Power to Question

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 tumour 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 abberations appear to correlate with an inhibition of cyclin B-Cdk1 kinase activity, due to a protracted inhibitory phosphorylated state of Cdk1.

REFERENCES

- 1. Diella, F., et al. 1997. Characterization of the Int-6 mammary tumor gene product. DNA Cell Biol. 16: 839-847.
- Asano, K., et al. 1997. The translation initiation factor elF3-p48 subunit is encoded by Int-6, a site of frequent integration by the mouse mammary tumor virus genome. J. Biol. Chem. 272: 23477-23480.
- Guo, J., et al. 2000. Characterization of the interaction between the interferon-induced protein P56 and the Int-6 protein encoded by a locus of insertion of the mouse mammary tumor virus. J. Virol. 74: 1892-1899.
- 4. Morris-Desbois, C., et al. 2001. The human protein HSPC021 interacts with Int-6 and is associated with eukaryotic translation initiation factor 3. J. Biol. Chem. 276: 45988-45995.
- 5. Rasmussen, S.B., et al. 2001. Evidence for the transforming activity of a truncated Int-6 gene, *in vitro*. Oncogene 20: 5291-5301.
- Yen, H.C., et al. 2003. Int-6—a link between the proteasome and tumorigenesis. Cell Cycle 2: 81-83.
- 7. von Arnim, A.G. and Chamovitz, D.A. 2003. Protein homeostasis: a degrading role for Int-6/eIF3e. Curr. Biol. 13: R323-R325.
- 8. Watkins, S.J., et al. 2004. Cell cycle-related variation in subcellular localization of elF3e/Int-6 in human fibroblasts. Cell Prolif. 37: 149-160.

CHROMOSOMAL LOCATION

Genetic locus: Eif3e (mouse) mapping to 15 B3.2.

PRODUCT

Int-6 siRNA (m) 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 (m): sc-40562-SH and Int-6 shRNA (m) Lentiviral Particles: sc-40562-V as alternate gene silencing products.

For independent verification of Int-6 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-40562A, sc-40562B and sc-40562C.

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 (m) is recommended for the inhibition of Int-6 expression in mouse 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

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-lgG κ BP-HRP: sc-516102 or m-lgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz MarkerTM Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-lgG κ BP-FITC: sc-516140 or m-lgG 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 (m)-PR: sc-40562-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.