SANTA CRUZ BIOTECHNOLOGY, INC.

eIF6 siRNA (m): sc-77256

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The Power to Question

BACKGROUND

The initiation of protein synthesis in eukaryotic cells is regulated by interactions between protein initiation factors and RNA molecules. Eukaryotic initiation factors (eIFs) are utilized in a sequence of reactions that lead to 80S ribosomal assembly and, ultimately, translation. eIF6 (eukaryotic translation initiation factor 6) is also known as CAB, B2GCN homolog, $p27^{BBP}$ or B4 integrin interactor and is a 245 amino acid protein that is localized to the cytoplasm, as well as to the nucleolus within the nucleus. The eIF6 N-terminal and C-terminal subdomains are thought to contain important nucleolar localization sequences. eIF6 may be a regulator of ribosomal function and creation. eIF6 functions to bind and translocate the 60S ribosomal subunit from the nucleus to the cytoplasm, effectively preventing the 60S subunit from associating with the 40S subunit and inhibiting formation of the 80S initiation complex. The regulation of the formation of the 80S ribosomes also regulates transcription. Once translocated to the cytoplasm, the eIF6-60S ribosomal subunit complex is subject to phosphorylation via the RACK1/PKC pathway, an event that results in the dissociation of eIF6 from the 60S subunit. Up-regulation of eIF6 is strongly associated with a variety of of cancers, such as ovarian cancer, suggesting that eIF6 may be involved in carcinogenesis.

REFERENCES

1. Groft, C.M., et al. 2000. Crystal structures of ribosome anti-association factor IF6. Nat. Struct. Biol. 7: 1156-1164.
2. Basu, U., et al. 2001. The *Saccharomyces cerevisiae* TIF6 gene encoding translation initiation factor 6 is required for 60S ribosomal subunit biogenesis. Mol. Cell. Biol. 21: 1453-1462.
3. Carotenuto, R., et al. 2005. Phosphorylation of $p27^{BBP}$/eIF6 and its association with the cytoskeleton are developmentally regulated in *Xenopus* oogenesis. Cell. Mol. Life Sci. 62: 1641-1652.
4. Balbo, A., et al. 2006. Cloning of *Dictyostelium* eIF6 ($p27^{BBP}$) and mapping its nucle(ol)ar localization subdomains. Eur. J. Cell Biol. 85: 1069-1078.
5. Donadini, A., et al. 2006. GABP complex regulates transcription of eIF6 ($p27^{BBP}$), an essential *trans*-acting factor in ribosome biogenesis. FEBS Lett. 580: 1983-1987.
6. Gandin, V., et al. 2008. Eukaryotic initiation factor 6 is rate-limiting in translation, growth and transformation. Nature 455: 684-688.

CHROMOSOMAL LOCATION

Genetic locus: Eif6 (mouse) mapping to 2 H1.

PRODUCT

eIF6 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see eIF6 shRNA Plasmid (m): sc-77256-SH and eIF6 shRNA (m) Lentiviral Particles: sc-77256-V as alternate gene silencing products.

For independent verification of eIF6 (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-77256A, sc-77256B and sc-77256C.

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

eIF6 siRNA (m) is recommended for the inhibition of eIF6 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

eIF6 (A-2): sc-390432 is recommended as a control antibody for monitoring of eIF6 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 eIF6 gene expression knockdown using RT-PCR Primer: eIF6 (m)-PR: sc-77256-PR (20 µl). 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.