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SANTA CRUZ BIOTECHNOLOGY, INC.

SBP-2 siRNA (m): sc-153236

BACKGROUND

Eukaryotes require a selenocysteine (Sec) insertion sequence (SECIS) element in the 3' untranslated region of the mRNA to decode the UGA codon as Sec. SECIS-binding protein 2 (SBP-2) specifically binds selenoprotein mRNAs to form a functional complex and is essential for the insertion of Sec into selenoproteins. Purified SBP-2 interacts specifically with the SECIS element in the phospholipid hydroperoxide glutathione peroxidase mRNA. SBP-2 shows binding activity in the liver and testis as well as hepatoma cells. SBP-2 binds to a conserved RNA binding domain shared with several ribosomal proteins and eukaryotic translation termination release factor 1. A second domain located N-terminal to the RNA binding domain required for Sec insertion allows SBP-2 to stably associate with the ribosomal fraction of cells. SBP-2 preferentially stimulates incorporation directed by the selenoprotein P and phospholipid hydroperoxide glutathione peroxidase SECIS elements. SBP-2 may have a distinct function in selecting the ribosomes for Sec insertion.

REFERENCES

1. Berry, M.J., et al. 1991. Recognition of UGA as a selenocysteine codon in type 1 deiodinase requires sequencs in the 3' untranslated region. Nature 353: 273-276.
2. Lesoon, A., et al. 1997. An RNA-binding protein recognizes a mammalian selenocysteine insertion sequence element required for cotranslational incorporation of selenocysteine. Mol. Cell. Biol. 17: 1977-1985.
3. Copeland, P.R. and Driscoll, D.M. 1999. Purification, redox sensitivity, and RNA binding properties of SECIS-binding protein 2, a protein involved in selenoprotein biosynthesis. J. Biol. Chem. 274: 25447-25454.
4. Copeland, P., et al. 2000. A novel RNA binding protein, SBP-2, is required for the translation of mammalian selenoprotein mRNAs. EMBO J. 19: 306-314.
5. Low, S.C., et al. 2000. SECIS-SBP-2 interactions dictate selenocysteine incorporation efficiency and selenoprotein hierarchy. EMBO J. 19: 6882-6890.
6. Copeland, P.R., et al. 2001. Insight into mammmalian selenocysteine insertion: domain structure and ribosome binding properties of Sec insertion sequence binding protein 2. Mol. Cell. Biol. 21: 1491-1498.

CHROMOSOMAL LOCATION

Genetic locus: Secisbp2 (mouse) mapping to 13 A5.

PRODUCT

SBP-2 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 SBP-2 shRNA Plasmid (m): sc-153236-SH and SBP-2 shRNA (m) Lentiviral Particles: sc-153236-V as alternate gene silencing products.

For independent verification of SBP-2 (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-153236A, sc-153236B and sc-153236C.

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

SBP-2 siRNA (m) is recommended for the inhibition of SBP-2 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

SBP-2 (C-10): sc-393651 is recommended as a control antibody for monitoring of SBP-2 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 SBP-2 gene expression knockdown using RT-PCR Primer: SBP-2 (m)-PR: sc-153236-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.