SANTA CRUZ BIOTECHNOLOGY, INC.

E6BP siRNA (h): sc-62247

BACKGROUND

E6BP (reticulocalbin 2, EF-hand calcium binding domain), also known as Rcn2, reticulocalbin-2, TCBP-49, taipoxin-associated calcium-binding protein 49, Erc55 or calcium-binding protein ERC-55, is a 320 amino acid protein with a high affinity for calcium that belongs to the CREC family. Encoded by a gene that maps to human chromosome 15q24.3, E6BP binds calcium ions and the presynaptic snake venom toxin taipoxin and may interact with internalized taipoxin, possibly mediating its activation. E6BP is expressed in rat brain, cerebellum, heart, liver, kidney, skeletal muscle and testis cells. Although E6BP is homologous to other known S100 proteins and EF-hand proteins, such as calmodulin and calbindin, homolgy is limited to the EF-hand domain. E6BP is also homologous to an identified mouse ER (endoplasmic reticulum) calcium binding protein, reticulocalbin, where homology is not restricted to the EF-hand domain. E6BP is not an abundant protein, which suggests that it is not a reticular calcium storage protein. E6BP is believed to share a general E6-binding motif with E6-AP, which binds cancer-related papillomaviral E6 proteins.

REFERENCES

1. Dodds, D., et al. 1995. Novel reticular calcium binding protein is purified on taipoxin columns. J. Neurochem. 64: 2339-2344.
2. Be, X., et al. 2001. Solution structure determination and mutational analysis of the papillomavirus E6 interacting peptide of E6AP. Biochemistry 40: 1293-1299.
3. Nakagawa, M., et al. 2002. Overexpression of RCN1 and RCN2, rice TERMINAL FLOWER 1/CENTRORADIALIS homologs, confers delay of phase transition and altered panicle morphology in rice. Plant J. 29: 743-750.
4. Sherman, L., et al. 2002. Inhibition of serum- and calcium-induced terminal differentiation of human keratinocytes by HPV 16 E6: study of the association with p53 degradation, inhibition of p53 transactivation, and binding to E6BP. Virology 292: 309-320.
5. Zanier, K., et al. 2005. Kinetic analysis of the interactions of human papillomavirus E6 oncoproteins with the ubiquitin ligase E6AP using surface plasmon resonance. J. Mol. Biol. 349: 401-412.

CHROMOSOMAL LOCATION

Genetic locus: RCN2 (human) mapping to 15q24.3.

PRODUCT

E6BP siRNA (h) 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 E6BP shRNA Plasmid (h): sc-62247-SH and E6BP shRNA (h) Lentiviral Particles: sc-62247-V as alternate gene silencing products.

For independent verification of E6BP (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-62247A, sc-62247B and sc-62247C.

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

E6BP siRNA (h) is recommended for the inhibition of E6BP expression in human 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

E6BP (30): sc-293069 is recommended as a control antibody for monitoring of E6BP 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 E6BP gene expression knockdown using RT-PCR Primer: E6BP (h)-PR: sc-62247-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.