SANTA CRUZ BIOTECHNOLOGY, INC.

ASB-9 shRNA (m) Lentiviral Particles: sc-141293-V

BACKGROUND

Members of the suppressor of cytokine signaling (SOCS) family of proteins contain C-terminal regions of homology called the SOCS box, which serves to couple SOCS proteins and their binding partners with the Elongin B and C complex. Several other families of proteins also contain SOCS boxes, but differ from the SOCS proteins in the type of domain they contain upstream of the SOCS box. The largest family of SOCS box-containing proteins is the ankyrin repeat and SOCS box-containing (ASB) protein family. Members of the ASB family include ASB-1 through ASB-18 and are involved in a variety of biological processes. ASB-9 is a 294 amino acid member of this family. It contains six ankyrin repeats and one SOCS box domain. ASB-9 functions as a ubiquitin ligase and, via its SOCS box domain, it specifically interacts with creatine kinase-B, targeting it for degradation and regulating its expression within the cell. Two isoforms exist for ASB-9 due to alternative splicing events.

REFERENCES

1. Bork, P. 1993. Hundreds of ankyrin-like repeats in functionally diverse proteins: mobile modules that cross phyla horizontally? Proteins 17: 363-374.
2. Hilton, D.J., et al. 1998. Twenty proteins containing a C-terminal SOCS box form five structural classes. Proc. Natl. Acad. Sci. USA 95: 114-119.
3. Kile, B.T., et al. 2000. Cloning and characterization of the genes encoding the ankyrin repeat and SOCS box-containing proteins ASB-1, ASB-2, ASB-3 and ASB-4. Gene 258: 331-341.
4. Kile, B.T., et al. 2002. The SOCS box: a tale of destruction and degradation. Trends Biochem. Sci. 27: 235-241.
5. Kohroki, J., et al. 2005. ASB proteins interact with Cullin5 and Rbx2 to form E3 ubiquitin ligase complexes. FEBS Lett. 579: 6796-6802.
6. Debrincat, M.A., et al. 2007. Ankyrin repeat and suppressors of cytokine signaling box protein ASB-9 targets creatine kinase-B for degradation. J. Biol. Chem. 282: 4728-4737.

CHROMOSOMAL LOCATION

Genetic locus: Asb9 (mouse) mapping to X F5.

PRODUCT

ASB-9 shRNA (m) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ASB-9 siRNA (m): sc-141293 and ASB-9 shRNA Plasmid (m): sc-141293-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

ASB-9 shRNA (m) Lentiviral Particles is recommended for the inhibition of ASB-9 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

ASB-9 (E-4): sc-166737 is recommended as a control antibody for monitoring of ASB-9 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 ASB-9 gene expression knockdown using RT-PCR Primer: ASB-9 (m)-PR: sc-141293-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.