SANTA CRUZ BIOTECHNOLOGY, INC.

SPOP siRNA (m): sc-63057

BACKGROUND

SPOP (speckle-type POZ protein), also known as TEF2, HIB homolog 1 or road-kill homolog 1, is a member of the Tdpoz family containing one N-terminal MATH (meprin and TRAF homology) domain and one C-terminal BTB/ POZ domain. SPOP can exist as a homodimer and is expressed in a variety of tissues localizing to the nucleus. Through an interaction with CUL-3, SPOP is involved in ubiquitinylation and protein degradation. SPOP specifically interacts with CUL-3 via its BTB/POZ domain and recruits substrates to the CUL-3-based ubiquitin ligase via its MATH domain. Substrates recruited by SPOP and targeted for ubiquitylation via the CUL-3/SPOP complex include PDX-1, Bmi-1, MacroH2A, PIPK II β and Daxx. These substrates are subsequently degraded by the proteasome. In addition, SPOP itself becomes ubiquitylated by the CUL-3-based ubiquitin ligase and is targeted for proteasomal degradation.

REFERENCES

1. Nagai, Y., et al. 1997. Identification of a novel nuclear speckle-type protein, SPOP. FEBS Lett. 418: 23-26.
2. Zapata, J.M., et al. 2001. A diverse family of proteins containing tumor necrosis factor receptor-associated factor domains. J. Biol. Chem. 276: 24242-24252.
3. Takahashi, I., et al. 2002. MacroH2A1.2 binds the nuclear protein SPOP. Biochim. Biophys. Acta 1591: 63-68.
4. Liu, A., et al. 2004. Identification of PCIF1, a POZ domain protein that inhibits PDX-1 (MODY4) transcriptional activity. Mol. Cell. Biol. 24: 4372-4383.
5. La, M., et al. 2004. Daxx-mediated transcriptional repression of MMP1 gene is reversed by SPOP. Biochem. Biophys. Res. Commun. 320: 760-765.
6. Hernández-Muñoz, I., et al. 2005. Stable X chromosome inactivation involves the PRC1 Polycomb complex and requires histone MACROH2A1 and the CULLIN3/SPOP ubiquitin E3 ligase. Proc. Natl. Acad. Sci. USA 102: 7635-7640.

CHROMOSOMAL LOCATION

Genetic locus: Spop (mouse) mapping to 11 D.

PRODUCT

SPOP 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 SPOP shRNA Plasmid (m): sc-63057-SH and SPOP shRNA (m) Lentiviral Particles: sc-63057-V as alternate gene silencing products.

For independent verification of SPOP (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-63057A, sc-63057B and sc-63057C.

PROTOCOLS

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

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

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

SPOP (B-8): sc-377206 is recommended as a control antibody for monitoring of SPOP 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 SPOP gene expression knockdown using RT-PCR Primer: SPOP (m)-PR: sc-63057-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.