

ASC siRNA (m): sc-37282

BACKGROUND

Caspase-associated recruitment domains (CARDs) mediate the interaction between adaptor proteins such as Apaf-1 and the proform of caspases (e.g., CASP9) participating in apoptosis. ASC (apoptosis-associated speck-like protein containing a CARD, also known as TMS1 or PYCARD) is a member of the CARD-containing adaptor protein family. ASC is a 195 amino acid protein, containing a N-terminal Pyrin-like domain (PYD) and an 87 residue C-terminal CARD. This motif is characteristic of numerous proteins involved in apoptotic signaling. Fluorescence microscopy demonstrates a ring-like expression in some transfected cells. Immunofluorescence microscopy demonstrates that induction of apoptosis causes a CARD-dependent shift from diffuse cytoplasmic expression to punctate or spherical perinuclear aggregates. Western blot analysis shows expression of ASC in leukemia and melanoma cell lines. ASC exhibits intriguing behavior by forming an aggregate and appearing as a speck during apoptosis induced by retinoic acid and other anti-tumor drugs. The ASC gene maps to human chromosome 16p11.2.

REFERENCES

1. Masumoto, J., et al. 1999. ASC, a novel 22 kDa protein, aggregates during apoptosis of human promyelocytic leukemia HL-60 cells. *J. Biol. Chem.* 274: 33835-33838.
2. Conway, K.E., et al. 2000. TMS1, a novel proapoptotic caspase recruitment domain protein, is a target of methylation-induced gene silencing in human breast cancers. *Cancer Res.* 60: 6236-6242.
3. McConnell, B.B., et al. 2000. Activation of a caspase-9-mediated apoptotic pathway by subcellular redistribution of the novel caspase recruitment domain protein TMS1. *Cancer Res.* 60: 6243-6247.

CHROMOSOMAL LOCATION

Genetic locus: Pycard (mouse) mapping to 7 F3.

PRODUCT

ASC 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ASC shRNA Plasmid (m): sc-37282-SH and ASC shRNA (m) Lentiviral Particles: sc-37282-V as alternate gene silencing products.

For independent verification of ASC (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-37282A, sc-37282B and sc-37282C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

ASC siRNA (m) is recommended for the inhibition of ASC 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 μ M in 66 μ l. 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

ASC (B-3): sc-514414 is recommended as a control antibody for monitoring of ASC 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ASC gene expression knockdown using RT-PCR Primer: ASC (m)-PR: sc-37282-PR (20 μ l, 400 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ren, S. and Chen, H. 2013. Regulatory effects of apoptosis-associated speck-like protein on cytokines in the P388D1 macrophage-like cell line. *Mol. Med. Rep.* 7: 166-170.
2. Xu, J., et al. 2014. Macrophage endocytosis of high-mobility group box 1 triggers pyroptosis. *Cell Death Differ.* 21: 1229-1239.
3. Zhang, Y., et al. 2015. Epidermal fatty acid binding protein promotes skin inflammation induced by high-fat diet. *Immunity* 42: 953-964.
4. Molagoda, I.M.N., et al. 2019. Deoxynivalenol enhances IL-1 β expression in BV2 microglial cells through activation of the NF κ B pathway and the ASC/NLRP3 inflammasome. *EXCLI J.* 18: 356-369.
5. Zhi, X., et al. 2019. NLRP3 inflammasome activation by foot-and-mouth disease virus infection mainly induced by viral RNA and non-structural protein 2B. *RNA Biol.* 17: 335-349.
6. Wang, X., et al. 2020. PCSK9 regulates pyroptosis via mtDNA damage in chronic myocardial ischemia. *Basic Res. Cardiol.* 115: 66.

RESEARCH USE

For research use only, not for use in diagnostic procedures.