

# CARD 12 siRNA (h): sc-60328

## BACKGROUND

Membrane-associated guanylate kinase (MAGUK) family members localize to the plasma membrane and function as molecular scaffolds for the assembly of multi-protein complexes. The MAGUK family includes several mammalian proteins related to the *Drosophila* tumor suppressor discs-large (dlg) gene product such as postsynaptic proteins, GKAPs, the tight junction associated proteins (ZO-1-3) and the caspase-associated recruitment domain (CARD) proteins: CARD 6, CARD 8-12 and CARD 14. CARD 12 is expressed at high levels in bone marrow and expressed at lower levels in lymph node, placenta, spleen and brain tissues. CARD 12 regulates the activation of caspase-1, a caspase that plays a role in both apoptotic signaling and cytokine processing. The nucleotide-binding site domain of CARD 12 is specific for ATP/dATP. CARD 12 associates with itself and with ASC, an associated speck-like protein containing a CARD, recently identified as a proapoptotic protein. Together, they induce apoptosis and inflammatory signaling pathways.

## REFERENCES

1. Geddes, B.J., et al. 2001. Human CARD 12 is a novel CED4/Apaf-1 family member that induces apoptosis. *Biochem. Biophys. Res. Commun.* 284: 77-82.
2. Online Mendelian Inheritance in Man, OMIM<sup>™</sup>. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606831. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Damiano, J.S., et al. 2004. Multiple roles of CLAN (caspase-associated recruitment domain, leucine-rich repeat and NAIP CIA HET-E and TP1-containing protein) in the mammalian innate immune response. *J. Immunol.* 173: 6338-6345.

## CHROMOSOMAL LOCATION

Genetic locus: NLRC4 (human) mapping to 2p22.3.

## PRODUCT

CARD 12 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CARD 12 shRNA Plasmid (h): sc-60328-SH and CARD 12 shRNA (h) Lentiviral Particles: sc-60328-V as alternate gene silencing products.

For independent verification of CARD 12 (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-60328A, sc-60328B and sc-60328C.

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

## APPLICATIONS

CARD 12 siRNA (h) is recommended for the inhibition of CARD 12 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  $\mu$ M in 66  $\mu$ 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

CARD 12 (F-3): sc-514658 is recommended as a control antibody for monitoring of CARD 12 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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CARD 12 gene expression knockdown using RT-PCR Primer: CARD 12 (h)-PR: sc-60328-PR (20  $\mu$ l, 581 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Luo, X., et al. 2012. Docosahexaenoic acid ameliorates palmitate-induced lipid accumulation and inflammation through repressing NLRC4 inflammasome activation in Hep G2 cells. *Nutr. Metab.* 9: 34.
2. Grandjean, T., et al. 2017. The human NAIP-NLRC4-inflammasome senses the *Pseudomonas aeruginosa* T3SS inner-rod protein. *Int. Immunol.* 29: 377-384.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.