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CARD 11 shRNA (m) Lentiviral Particles: sc-44937-V

BACKGROUND

Modular protein interaction domains are an evolutionarily conserved protein contour feature in tertiary and quaternary protein folding that gives rise to a dynamic protein signaling network by mediating the assembly of encoded components into specific signaling complexes. Caspase-associated recruitment domain (CARD) proteins CARD11 and CARD14 are members of the membrane-associated guanylate kinase (MAGUK) family, a class of proteins that function as molecular scaffolds for the assembly of multiprotein complexes at the plasma membrane. The human CARD11 gene maps to chromosome 7p22 and encodes a 1,147 amino acid protein. The human CARD14 gene maps to chromosome 17q25 and encodes a 1004 amino acid protein. CARD11 and CARD14 can function as components of signaling pathways that lead to activation of the transcription factor NF- κ B. The CARD domains of CARD11 and CARD14 can specifically interact with Bcl10, a protein known to function as a positive regulator of cell apoptosis and NF- κ B activation.

REFERENCES

1. Inohara, N., et al. 1999. Nod1, an Apaf-1-like activator of caspase-9 and nuclear factor- κ B. *J. Biol. Chem.* 274: 14560-14567.
2. Pawson T., et al. 2000. Protein-protein interactions define specificity in signal transduction. *Genes Dev.* 14: 1027-1047.
3. Bertin, J., et al. 2001. CARD11 and CARD14 are novel caspase recruitment domain (CARD)/membrane-associated guanylate kinase (MAGUK) family members that interact with BCL10 and activate NF κ B. *J. Biol. Chem.* 276: 11877-11882.
4. Gaide, O., et al. 2001. Carma1, a CARD-containing binding partner of Bcl10, induces Bcl10 phosphorylation and NF κ B activation. *FEBS Lett.* 496: 121-127.

CHROMOSOMAL LOCATION

Genetic locus: Card11 (mouse) mapping to 5 G2.

PRODUCT

CARD 11 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 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 CARD 11 siRNA (m): sc-44937 and CARD 11 shRNA Plasmid (m): sc-44937-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.

PROTOCOLS

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

APPLICATIONS

CARD 11 shRNA (m) Lentiviral Particles is recommended for the inhibition of CARD 11 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

CARD 11 (N-20): sc-20458 is recommended as a control antibody for monitoring of CARD 11 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker[™] compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CARD 11 gene expression knockdown using RT-PCR Primer: CARD 11 (m)-PR: sc-44937-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.