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SANTA CRUZ BIOTECHNOLOGY, INC.

cGAS siRNA (m): sc-143253

BACKGROUND

The presence of foreign DNA in the cytoplasm induces an antiviral host immune response. DNA in the cytoplasm triggers the production of interferons by activating and synthesis of second messenger cyclic guanosine monophosphate-adenosine monophosphate (cyclic GMP-AMP, or cGAMP). cGAS (cyclic GMP-AMP synthase), also known as MB21D1 (Mab-21 domain containing 1), h-cGAS or C6orf150, is a 522 amino acid cytoplasmic nucleotidyltransferase that catalyzes the formation of cyclic GMP-AMP (cGAMP) from ATP and GTP. cGAS is suggested to have antiviral activity by acting as a key cytosolic DNA sensor. cGAS binds to cytosolic DNA, which leads to cGAMP synthesis and activation of TMEM173, thereby trigger type-I interferon production. Expressed in monocytic cell line THP1, cGAS exists as two alternatively spliced isoforms and is encoded by a gene located on human chromosome 6q13.

REFERENCES

1. Schoggins, J.W., et al. 2011. A diverse range of gene products are effectors of the type I interferon antiviral response. Nature 472: 481-485.
2. Diner, E.J., et al. 2013. The innate immune DNA sensor cGAS produces a noncanonical cyclic dinucleotide that activates human STING. Cell Rep. 3: 1355-1361.
3. Li, X., et al. 2013. Cyclic GMP-AMP synthase is activated by double-stranded DNA-induced oligomerization. Immunity 39: 1019-1031.
4. Civril, F., et al. 2013. Structural mechanism of cytosolic DNA sensing by cGAS. Nature 498: 332-337.
5. Kato, K., et al. 2013. Structural and functional analyses of DNA-sensing and immune activation by human cGAS. PLoS ONE 8: e76983.
6. Sun, L., et al. 2013. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 339: 786-791.
7. Kranzusch, P.J., et al. 2014. Structure-guided reprogramming of human cGAS dinucleotide linkage specificity. Cell 158: 1011-1021.
8. Zhang, X., et al. 2014. The cytosolic DNA sensor cGAS forms an oligomeric complex with DNA and undergoes switch-like conformational changes in the activation loop. Cell Rep. 6: 421-430.

CHROMOSOMAL LOCATION

Genetic locus: Mb21d1 (mouse) mapping to 9 E1.

PRODUCT

cGAS 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 cGAS shRNA Plasmid (m): sc-143253-SH and cGAS shRNA (m) Lentiviral Particles: sc-143253-V as alternate gene silencing products.

For independent verification of cGAS (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-143253A, sc-143253B and sc-143253C.

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

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

cGAS (D-9): sc-515777 is recommended as a control antibody for monitoring of cGAS 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 cGAS gene expression knockdown using RT-PCR Primer: cGAS (m)-PR: sc-143253-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.

PROTOCOLS

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