

cGAS siRNA (h): sc-95512

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

- 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.
- 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.
- Li, X., et al. 2013. Cyclic GMP-AMP synthase is activated by double-stranded DNA-induced oligomerization. *Immunity* 39: 1019-1031.

CHROMOSOMAL LOCATION

Genetic locus: MB21D1 (human) mapping to 6q13.

PRODUCT

cGAS siRNA (h) is a pool of 2 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 cGAS shRNA Plasmid (h): sc-95512-SH and cGAS shRNA (h) Lentiviral Particles: sc-95512-V as alternate gene silencing products.

For independent verification of cGAS (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-95512A and sc-95512B.

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

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

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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 (h)-PR: sc-95512-PR (20 μ l, 491 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Suspène, R., et al. 2017. Self-cytoplasmic DNA upregulates the mutator enzyme APOBEC3A leading to chromosomal DNA damage. *Nucleic Acids Res.* 45: 3231-3241.
- Liu, H., et al. 2018. Nuclear cGAS suppresses DNA repair and promotes tumorigenesis. *Nature* 563: 131-136.
- Davis, S.E., et al. 2019. Nucleosomal dsDNA stimulates APOL1 expression in human cultured podocytes by activating the cGAS/IFI16-STING signaling pathway. *Sci. Rep.* 9: 15485.
- Uhlorn, B.L., et al. 2020. Attenuation of cGAS/STING activity during mitosis. *Life Sci. Alliance* 3: e201900636.
- Sheikh, T.N., et al. 2021. Growth inhibition and induction of innate immune signaling of chondrosarcomas with epigenetic inhibitors. *Mol. Cancer Ther.* 20: 2362-2371.

RESEARCH USE

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