SANTA CRUZ BIOTECHNOLOGY, INC.

G9a siRNA (h): sc-43777

BACKGROUND

Distinct modifications of histone tails, such as acetylation, phosphorlation and methylation, regulate nuclear processes, such as control of transcription and mitotic chromosome condensation. Histone methyltransferases (HMTases) are among the different groups of enzymes known to catalyze the covalent modification. G9a, a SET domain-containing protein, is a novel mammalian lysine-preferring HMTase. G9a, also known as BAT8, NG36 or HMTase (for mammalian histone methyltransferase), has strong HMTase activity towards Histone H3 lysine 9 methylation *in vitro*. G9a plays a dominant role in euchromatic Histone H3 lysine 9 methylation, is essential for early embryogenesis and is involved in the transcriptional repression of developmental genes. Like SUV39H, G9a transfers methyl groups to the lysine residues of Histone H3, but with a 10-20-fold higher activity than SUV39H1. G9a also adds methyl groups to lysine 27 as well as lysine 9 in Histone H3. G9a localizes in the nucleus, indicating that it may contribute to the organization of the higher order chromatin structure of non-centromeric loci. The human G9a gene maps to chromosome 6p21.33.

CHROMOSOMAL LOCATION

Genetic locus: EHMT2 (human) mapping to 6p21.33.

PRODUCT

G9a 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see G9a shRNA Plasmid (h): sc-43777-SH and G9a shRNA (h) Lentiviral Particles: sc-43777-V as alternate gene silencing products.

For independent verification of G9a (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-43777A, sc-43777B and sc-43777C.

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

G9a siRNA (h) is recommended for the inhibition of G9a expression in human cells.

PROTOCOLS

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

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

G9a (C-9): sc-515726 is recommended as a control antibody for monitoring of G9a 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 G9a gene expression knockdown using RT-PCR Primer: G9a (h)-PR: sc-43777-PR (20 µl, 419 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. El Gazzar, M., et al. 2008. G9a and HP1 couple histone and DNA methylation to TNFα transcription silencing during endotoxin tolerance. J. Biol. Chem. 283: 32198-32208.
2. Li, Q., et al. 2010. Polycomb CBX7 directly controls trimethylation of histone H3 at lysine 9 at the p16 locus. PLoS ONE 5: e13732.
3. Choi, J.D., et al. 2012. Suppression and recovery of BRCA1-mediated transcription by HP1γ via modulation of promoter occupancy. Nucleic Acids Res. 40: 11321-11338.
4. Kim, Y., et al. 2013. BIX-01294 induces autophagy-associated cell death via EHMT2/G9a dysfunction and intracellular reactive oxygen species production. Autophagy 9: 2126-2139.
5. Chen, X., et al. 2014. The visualization of large organized chromatin domains enriched in the H3K9me2 mark within a single chromosome in a single cell. Epigenetics 9: 1439-1445.
6. Paschall, A.V., et al. 2015. H3K9 trimethylation silences FAS expression to confer colon carcinoma immune escape and 5-fluorouracil chemoresistance. J. Immunol. 195: 1868-1882.
7. Park, S.E., et al. 2016. Inhibition of EHMT2/G9a epigenetically increases the transcription of Beclin-1 via an increase in ROS and activation of NFκB. Oncotarget 7: 39796-39808.
8. Chen, R.J., et al. 2017. Methyltransferase G9a promotes cervical cancer angiogenesis and decreases patient survival. Oncotarget 8: 62081-62098.

RESEARCH USE

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