SANTA CRUZ BIOTECHNOLOGY, INC.

Histone H2A.X siRNA (h): sc-62464

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The Power to Question

BACKGROUND

Histone H2A.X is a member of the Histone H2A family, which is involved in nucleosomal organization of chromatin. The H2AFX gene is located in close proximity to the porphobilinogen deaminase (PBGD) gene in both mouse and human, and maps to chromosome 9 and 11q23.3, respectively. H2A.X differs from the other members of the H2A family by the presence of a highly conserved C-terminal motif. It is rapidly phosphorylated in response to ionizing radiation and plays an important role in the recognition and repair of DNA double stranded breaks. The phosphorylated form of H2A.X, designated γ-H2A.X, forms nuclear foci at the heavy chain constant region of cells involved in class switch recombination (CSR), a region-specific DNA reaction that replaces one immunoglobulin heavy chain constant region gene with another. The phosphorylated γ-H2A.X is also thought to initiate subsequent repair factors, including Rad50, Rad51 and BRCA1.

CHROMOSOMAL LOCATION

Genetic locus: H2AFX (human) mapping to 11q23.3.

PRODUCT

Histone H2A.X siRNA (h) is a target-specific 19-25 nt siRNA 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 Histone H2A.X shRNA Plasmid (h): sc-62464-SH and Histone H2A.X shRNA (h) Lentiviral Particles: sc-62464-V as alternate gene silencing products.

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

Histone H2A.X siRNA (h) is recommended for the inhibition of Histone H2A.X 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 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

Histone H2A.X (938CT5.1.1): sc-517336 is recommended as a control antibody for monitoring of Histone H2A.X 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 Histone H2A.X gene expression knockdown using RT-PCR Primer: Histone H2A.X (h)-PR: sc-62464-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Malla, R.R., et al. 2012. uPAR and cathepsin B inhibition enhanced radiation-induced apoptosis in gliomainitiating cells. Neuro Oncol. 14: 745-760.
2. Dong, Y., et al. 2014. H2AX phosphorylation regulated by p38 is involved in Bim expression and apoptosis in chronic myelogenous leukemia cells induced by imatinib. Apoptosis 19: 1281-1292.
3. Wu, X.P., et al. 2015. Resveratrol induces apoptosis of human chronic myelogenous leukemia cells *in vitro* through p38 and JNK-regulated H2AX phosphorylation. Acta Pharmacol. Sin. 36: 353-361.
4. Wen, L., et al. 2016. Regulation of multi-drug resistance in hepatocellular carcinoma cells is TRPC6/calcium dependent. Sci. Rep. 6: 23269.
5. Yin, H., et al. 2019. Actinomycin D-activated RNase L promotes H2A.X/H2B-mediated DNA damage and apoptosis in lung cancer cells. Front. Oncol. 9: 1086.
6. Kawashima, S., et al. 2020. γ-H2AX as a potential indicator of radiosensitivity in colorectal cancer cells. Oncol. Lett. 20: 2331-2337.

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.