SANTA CRUZ BIOTECHNOLOGY, INC.

Histone H2A.X siRNA (m): sc-62465

SANTA CRUZ BIOTECHNOLOGY

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.

REFERENCES

1. Ivanova, V.S., et al. 1994. Characterization of the human histone H2A.X gene: comparison of its promoter with other H2A gene promoters. J. Biol. Chem. 269: 24189-24194.
2. Porcher, C. and Grandchamp, B. 1995. Structure of the mouse H2A.X gene and physical linkage to the UPS locus on chromosome 9: assignment of the human H2A.X gene to 11q23 by sequence anyalysis. Genomics 25: 312-313.
3. Paull, T.T., et al. 2000. A critical role for Histone H2A.X in recruitment of repair factors to nuclear foci after DNA damage. Curr. Biol. 10: 886-895.
4. Rogakou, E.P., et al. 2000. Initiation of DNA fragmentation during apoptosis induces phosphorylation of Histone H2A.X at Serine 139. J. Biol. Chem. 275: 9390-9395.
5. Petersen, S., et al. 2001. AID is required to initiate NBS1/γ-H2A.X focus formation and mutations at sites of class switching. Nature 414: 660-665.
6. Ward, I.M. and Chen, J. 2001. Histone H2A.X is phosphorylated in an ATR-dependent manner in response to replicational stress. J. Biol. Chem. 276: 47759-47762.

CHROMOSOMAL LOCATION

Genetic locus: H2afx (mouse) mapping to 9 A5.2.

PRODUCT

Histone H2A.X 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 Histone H2A.X shRNA Plasmid (m): sc-62465-SH and Histone H2A.X shRNA (m) Lentiviral Particles: sc-62465-V as alternate gene silencing products.

For independent verification of Histone H2A.X (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-62465A, sc-62465B and sc-62465C.

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

p-Histone H2A.X (Ser 139): sc-517348 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).

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 (m)-PR: sc-62465-PR (20 µl, 556 bp). 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.