SANTA CRUZ BIOTECHNOLOGY, INC.

MacroH2A siRNA (h): sc-62575

BACKGROUND

Eukaryotic histones are water soluble, basic nuclear proteins that form hetero-octameric nucleosome particles by wrapping 146 base pairs of DNA in a left-handed super-helical turn sequentially to form chromosomal fiber. Two molecules of each of the four core histones (H2A, H2B, H3, and H4) form the octamer. The octamer consists of two H2A-H2B dimers and two H3-H4 dimers, forming two nearly symmetrical halves by tertiary structure. MacroH2A, also called core histone MacroH2A2 (mH2A2), is a variant Histone H2A, originally isolated in rat liver, that is nearly three times as large as conventional H2A. MacroH2A may be involved in stable X chromosome inactivation as it is enriched in inactive X chromosome chromatin.

REFERENCES

1. Pehrson, J.R., et al. 1992. MacroH2A, a core histone containing a large nonhistone region. Science 257: 1398-1400.
2. Chadwick, B.P., et al. 2001. Histone H2A variants and the inactive X chromosome: identification of a second MacroH2A variant. Hum. Mol. Genet. 10: 1101-1113.
3. Costanzi, C., et al. 2001. MacroH2A2, a new member of the MacroH2A core histone family. J. Biol. Chem. 276: 21776-21784.
4. Chakravarthy, S., et al. 2005. Structural characterization of the histone variant MacroH2A. Mol. Cell. Biol. 25: 7616-7624.
5. Kustatscher, G., et al. 2005. Splicing regulates NAD metabolite binding to histone MacroH2A. Nat. Struct. Mol. Biol. 12: 624-625.
6. Ma, Y., et al. 2005. DNA CpG hypomethylation induces heterochromatin reorganization involving the histone variant MacroH2A. J. Cell Sci. 118: 1607-1616.
7. Hernández-Muñoz, I., et al. 2005. Stable X chromosome inactivation involves the PRC1 Polycomb complex and requires histone MacroH2A1 and the CULLIN3/SPOP ubiquitin E3 ligase. Proc. Natl. Acad. Sci. USA 102: 7635-7640.
8. Chu, F., et al. 2006. Mapping post-translational modifications of the histone variant MacroH2A1 using tandem mass spectrometry. Mol. Cell. Proteomics 5: 194-203.

CHROMOSOMAL LOCATION

Genetic locus: H2AFY2 (human) mapping to 10q22.1.

PRODUCT

MacroH2A 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 MacroH2A shRNA Plasmid (h): sc-62575-SH and MacroH2A shRNA (h) Lentiviral Particles: sc-62575-V as alternate gene silencing products.

For independent verification of MacroH2A (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-62575A, sc-62575B and sc-62575C.

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

MacroH2A siRNA (h) is recommended for the inhibition of MacroH2A 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

MacroH2A (C-9): sc-377452 is recommended as a control antibody for monitoring of MacroH2A 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 MacroH2A gene expression knockdown using RT-PCR Primer: MacroH2A (h)-PR: sc-62575-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.