SANTA CRUZ BIOTECHNOLOGY, INC.

DNA-PK$_{CS}$ siRNA (m): sc-35201

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

The phosphatidylinositol kinase (PIK) family members fall into two distinct subgroups. The first subgroup contains proteins such as the PI 3- and PI 4-kinases and the second group comprises the PIK-related kinases. The PIK-related kinases include Atm, DNA-PK$_{CS}$ and FRAP. These proteins have in common a region of homology at their carboxy termini that is not present in the PI 3- and PI 4-kinases. The Atm gene is mutated in the autosomal recessive disorder ataxia telangiectasia (AT) that is characterized by cerebellar degeneration (ataxia) and the appearance of dilated blood vessels (telangiectases) in the conjunctivae of the eyes. AT cells are hypersensitive to ionizing radiation, impaired in mediating the inhibition of DNA synthesis and they display delays in p53 induction. DNA-PK is a heterotrimeric DNA binding enzyme that is composed of a large subunit, DNA-PK$_{CS}$, and two smaller subunits collectively known as Ku. The loss of DNA-PK leads to defects in DSB repair and V(D)J recombination. FRAP can autophosphorylate on serine and bind to rapamycin/FKBP. FRAP is also an upstream regulator of S6 kinase and has been implicated in the regulation of p27 and p21 expression.

REFERENCES

1. Nowak, R. 1995. Discovery of AT gene sparks biomedical research bonanza. Science 268: 1700-1701.
2. Savitsky, K., et al. 1995. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 268: 1749-1753.
3. Hartley, K.O., et al. 1995. DNA-dependent protein kinase catalytic subunit: a relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. Cell 82: 849-856.

CHROMOSOMAL LOCATION

Genetic locus: Prkdc (mouse) mapping to 16 A2.

PRODUCT

DNA-PK$_{CS}$ 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 DNA-PK$_{CS}$ shRNA Plasmid (m): sc-35201-SH and DNA-PK$_{CS}$ shRNA (m) Lentiviral Particles: sc-35201-V as alternate gene silencing products.

For independent verification of DNA-PK$_{CS}$ (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-35201A, sc-35201B and sc-35201C.

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

DNA-PK$_{CS}$ siRNA (m) is recommended for the inhibition of DNA-PK$_{CS}$ 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

DNA-PK$_{CS}$ (G-12): sc-390849 is recommended as a control antibody for monitoring of DNA-PK$_{CS}$ 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 DNA-PK$_{CS}$ gene expression knockdown using RT-PCR Primer: DNA-PK$_{CS}$ (m)-PR: sc-35201-PR (20 µl, 458 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Zhang, M., et al. 2018. Tumor cell-accelerated senescence is associated with DNA-PK$_{CS}$ status and telomere dysfunction induced by radiation. Dose Response 16: 1559325818771527.
2. Zhou, H., et al. 2018. Effects of melatonin on fatty liver disease: the role of NR4A1/DNA-PK$_{CS}$/p53 pathway, mitochondrial fission, and mitophagy. J. Pineal Res. E-published.
3. Tang, S., et al. 2019. Ionizing radiation-induced growth in soft agar is associated with miR-21 upregulation in wild-type and DNA double strand break repair deficient cells. DNA Repair 78: 37-44.

RESEARCH USE

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