SANTA CRUZ BIOTECHNOLOGY, INC.

mTOR siRNA (h): sc-35409

BACKGROUND

The PIK-related kinases include Atm, DNA-PK_{CS} and mTOR. The Atm gene is mutated in the autosomal recessive disorder ataxia telangiectasia (AT) that is characterized by cerebellar degeneration and the appearance of dilated blood vessels 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. mTOR, also known as FRAP, can autophosphorylate on serine and bind to rapamycin/FKBP. mTOR is also an upstream regulator of S6 kinase and has been implicated in the regulation of p27 and p21 expression. mTOR autophosphorylates at Ser2481 under translationally repressive conditions. Phosphorylation of mTOR at Ser2448 is mediated by p70S6 kinase.

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. Keith, C.T. and Schreiber, S.L. 1995. PIK-related kinases: DNA repair, recombination, and cell cycle checkpoints. Science 270: 50-51.

CHROMOSOMAL LOCATION

Genetic locus: MTOR (human) mapping to 1p36.22.

PRODUCT

mTOR 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 mTOR shRNA Plasmid (h): sc-35409-SH and mTOR shRNA (h) Lentiviral Particles: sc-35409-V as alternate gene silencing products.

For independent verification of mTOR (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-35409A, sc-35409B and sc-35409C.

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

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

p-mTOR (59.Ser 2448): sc-293133 is recommended as a control antibody for monitoring of mTOR 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 mTOR gene expression knockdown using RT-PCR Primer: mTOR (h)-PR: sc-35409-PR (20 µl, 428 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kong, D., et al. 2008. Mammalian target of rapamycin repression by 3,3'-diindolylmethane inhibits invasion and angiogenesis in platelet-derived growth factor-D-overexpressing PC3 cells. Cancer Res. 68: 1927-1934.
2. Chen, K.D., et al. 2014. Interconnections between autophagy and the coagulation cascade in hepatocellular carcinoma. Cell Death Dis. 5: e1244.
3. Hou, G., et al. 2014. Targeted inhibition of mTOR signaling improves sensitivity of esophageal squamous cell carcinoma cells to cisplatin. J. Immunol. Res. 2014: 845763.
4. Mukhopadhyay, S., et al. 2015. Reciprocal regulation of AMP-activated protein kinase and phospholipase D. J. Biol. Chem. 290: 6986-6993.
5. Lu, Q., et al. 2015. Akt inhibition attenuates rasfonin-induced autophagy and apoptosis through the glycolytic pathway in renal cancer cells. Cell Death Dis. 6: e2005.
6. Song, K.A., et al. 2018. Increased synthesis of MCL-1 protein underlies initial survival of EGFR-mutant lung cancer to EGFR inhibitors and provides a novel drug target. Clin. Cancer Res. 24: 5658-5672.
7. Wu, Y.F., et al. 2019. Inactivation of MTOR promotes autophagy-mediated epithelial injury in particulate matter-induced airway inflammation. Autophagy 16: 1-16.
8. Nanni, M., et al. 2019. The aberrant expression of the mesenchymal variant of FGFR2 in the epithelial context inhibits autophagy. Cells 8 pii: E653.

RESEARCH USE

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