

ATE1 siRNA (h): sc-60220

BACKGROUND

Arginyl-tRNA-protein transferase (ATE1), also designated arginyltransferase 1, belongs to the R-transferase family of proteins. In order for a protein to be degraded via the ubiquitin pathway, arginylation of the protein is required. ATE1 plays an important role in this process, as it is important for the post-translational conjugation of arginine to the N-terminal aspartate-, glutamate- and possibly cystine-containing substrates. ATE1 is a 518 amino acid protein. Alternative splicing results in two distinct isoforms. ATE1, which is found as a monomer, can localize to the cytoplasm and/or the nucleus.

REFERENCES

1. Kwon, Y.T., et al. 1999. Alternative splicing results in differential expression, activity, and localization of the two forms of arginyl-tRNA-protein transferase, a component of the N-end rule pathway. *Mol. Cell. Biol.* 19: 182-193.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607103. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. Kwon, Y.T., et al. 2002. An essential role of N-terminal arginylation in cardiovascular development. *Science* 297: 96-99.
4. Hu, R.G., et al. 2005. The N-end rule pathway as a nitric oxide sensor controlling the levels of multiple regulators. *Nature* 437: 981-986.
5. Rai, R., et al. 2005. Identification of mammalian arginyltransferases that modify a specific subset of protein substrates. *Proc. Natl. Acad. Sci. USA* 102: 10123-10128.
6. Lee, M.J., et al. 2005. RGS4 and RGS5 are *in vivo* substrates of the N-end rule pathway. *Proc. Natl. Acad. Sci. USA* 102: 15030-15035.

CHROMOSOMAL LOCATION

Genetic locus: ATE1 (human) mapping to 10q26.13.

PRODUCT

ATE1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ATE1 shRNA Plasmid (h): sc-60220-SH and ATE1 shRNA (h) Lentiviral Particles: sc-60220-V as alternate gene silencing products.

For independent verification of ATE1 (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-60220A, sc-60220B and sc-60220C.

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.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

ATE1 siRNA (h) is recommended for the inhibition of ATE1 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 μ M in 66 μ l. 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

ATE1 (G-6): sc-398805 is recommended as a control antibody for monitoring of ATE1 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 ATE1 gene expression knockdown using RT-PCR Primer: ATE1 (h)-PR: sc-60220-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.