

ECA39 siRNA (h): sc-77222

BACKGROUND

Class-IV pyridoxal-phosphate-dependent aminotransferase family members ECA39 and BCAT2 are both enzymes that catalyze the first reaction in the catabolism of the essential branched chain amino acids valine, leucine and isoleucine. ECA39, also known as BCAT1 (branched-chain-amino-acid aminotransferase 1, cytosolic) is localized to the cytoplasm where it forms a homodimer. ECA39 is expressed in the brain and kidney during embryogenesis and is overexpressed in c-Myc induced tumors. BCAT2 (branched-chain-amino-acid aminotransferase 2, mitochondrial), also known as placental protein 18 (PP18), is expressed as two isoforms produced by alternative splicing. The first isoform of BCAT2, designated BCAT2A, is expressed in the mitochondrion, while the second isoform, designated BCAT2B, is expressed in the cytoplasm. Ubiquitously expressed, BCAT2 is also thought to act as a transporter of branched chain α -keto acids.

REFERENCES

- Schuldiner, O., et al. 1996. ECA39, a conserved gene regulated by c-Myc in mice, is involved in G₁/S cell cycle regulation in yeast. *Proc. Natl. Acad. Sci. USA* 93: 7143-7148.
- Ben-Yosef, T., et al. 1998. Characterization of murine BCAT genes: BCAT1, a c-Myc target, and its homolog, BCAT2. *Mamm. Genome* 9: 595-597.
- Eden, A., et al. 1999. Involvement of branched-chain amino acid aminotransferase (BCAT1/ECA39) in apoptosis. *FEBS Lett.* 457: 255-261.
- Grimm, C.H., et al. 2003. Lrmp and BCAT1 are candidates for the type I diabetes susceptibility locus Idd6. *Autoimmunity* 36: 241-246.

CHROMOSOMAL LOCATION

Genetic locus: BCAT1 (human) mapping to 12p12.1.

PRODUCT

ECA39 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 ECA39 shRNA Plasmid (h): sc-77222-SH and ECA39 shRNA (h) Lentiviral Particles: sc-77222-V as alternate gene silencing products.

For independent verification of ECA39 (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-77222A, sc-77222B and sc-77222C.

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

ECA39 siRNA (h) is recommended for the inhibition of ECA39 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

ECA39 (1F8): sc-517185 is recommended as a control antibody for monitoring of ECA39 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 ECA39 gene expression knockdown using RT-PCR Primer: ECA39 (h)-PR: sc-77222-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Xu, M., et al. 2016. BCAT1 promotes tumor cell migration and invasion in hepatocellular carcinoma. *Oncol. Lett.* 12: 2648-2656.
- Ding, Y., et al. 2022. BCAT1, as a prognostic factor for HCC, can promote the development of liver cancer through activation of the AKT signaling pathway and EMT. *J. Mol. Histol.* E-published.

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.