SANTA CRUZ BIOTECHNOLOGY, INC.

ABAT siRNA (m): sc-140745

BACKGROUND

In the central nervous system GABA (γ-aminobutyric acid) functions as the main inhibitory transmitter by increasing a Cl^- conductance that inhibits neuronal firing. ABAT (4-aminobutyrate aminotransferase), also known as GABAT (GABA aminotransferase), L-AIBAT or (S)-3-amino-2-methylpropionate transaminase, is a 500 amino acid mitochondrial matrix protein belonging to the class-III pyridoxal-phosphate-dependent aminotransferase family, which catabolizes GABA into succinic semialdehyde. Existing as a homodimer, ABAT binds pyridoxal phosphate as a cofactor and is expressed in liver, brain, pancreas, kidney, placenta and heart. The gene encoding ABAT maps to human chromosome 16p13.2, and defects in ABAT are the cause of GABA-AT deficiency, which is characterized by hypotonia, hyperreflexia, psychomotor retardation, lethargy, EEG abnormalities and refractory seizures.

REFERENCES

1. Jeremiah, S., et al. 1981. The biochemical genetics of human γ-aminobutyric acid transaminase. Ann. Hum. Genet. 45: 231-236.
2. Jaeken, J., et al. 1984. γ-aminobutyric acid-transaminase deficiency: a newly recognized inborn error of neurotransmitter metabolism. Neuropediatrics 15: 165-169.
3. Bhattacharyya, S.P., et al. 1985. γ-aminobutyric acid transaminase (GABAT) polymorphism among ethnic groups in Singapore—with report of a new allele. Am. J. Hum. Genet. 37: 358-361.
4. Osei, Y.D., et al. 1995. Screening and sequence determination of a cDNA encoding the human brain 4-aminobutyrate aminotransferase. Gene 155: 185-187.
5. Medina-Kauwe, L.K., et al. 1999. 4-aminobutyrate aminotransferase (GABA-transaminase) deficiency. J. Inherit. Metab. Dis. 22: 414-427.
6. Online Mendelian Inheritance in Man, OMIM™. 2009. Johns Hopkins University, Baltimore, MD. MIM Number: 137150: World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: Abat (mouse) mapping to 16 A1.

PRODUCT

ABAT 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 ABAT shRNA Plasmid (m): sc-140745-SH and ABAT shRNA (m) Lentiviral Particles: sc-140745-V as alternate gene silencing products.

For independent verification of ABAT (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-140745A, sc-140745B and sc-140745C.

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 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

ABAT siRNA (m) is recommended for the inhibition of ABAT 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

ABAT (B-12): sc-393769 is recommended as a control antibody for monitoring of ABAT 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 ABAT gene expression knockdown using RT-PCR Primer: ABAT (m)-PR: sc-140745-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.