SANTA CRUZ BIOTECHNOLOGY, INC.

DAGLβ siRNA (h): sc-89591

BACKGROUND

Members of the AB hydrolase superfamily have diverse catalytic functions and play a crucial role in the metabolism of lipids. DAGLβ (diacylglycerol lipase β), also known as KCCR13L, is a 672 amino acid multi-pass membrane protein that belongs to the AB hydrolase superfamily. DAGLβ uses calcium as a cofactor to catalyze the hydrolysis of diacylglycerol (DAG) to 2-arachidonoyl-glycerol (2-AG), a reaction that is required for axonal growth and for retrograde synaptic signaling at mature synapses. DAGLβ functions at an optimal pH of 7 and its activity is inhibited by p-hydroxy-mercuri-benzoate and HgCl2, but not PMSF. There are three isoforms of DAGLβ that are produced as a result of alternative splicing events.

REFERENCES

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3. Bisogno, T., et al. 2003. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J. Cell Biol. 163: 463-468.
4. Ligresti, A., et al. 2005. Endocannabinoid metabolic pathways and enzymes. Curr. Drug Targets CNS Neurol. Disord. 4: 615-623.
5. Jung, K.M., et al. 2005. Stimulation of endocannabinoid formation in brain slice cultures through activation of group I metabotropic glutamate receptors. Mol. Pharmacol. 68: 1196-1202.
6. Hashimotodani, Y., et al. 2007. Endocannabinoids and synaptic function in the CNS. Neuroscientist 13: 127-137.
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CHROMOSOMAL LOCATION

Genetic locus: DAGLB (human) mapping to 7p22.1.

PRODUCT

DAGLβ 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 DAGLβ shRNA Plasmid (h): sc-89591-SH and DAGLβ shRNA (h) Lentiviral Particles: sc-89591-V as alternate gene silencing products.

For independent verification of DAGLβ (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-89591A, sc-89591B and sc-89591C.

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

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

DAGLβ (A-5): sc-514738 is recommended as a control antibody for monitoring of DAGLβ 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-IgGλ BP-HRP: sc-516132 or m-IgGλ BP-HRP (Cruz Marker): sc-516132-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-IgGλ BP-FITC: sc-516185 or m-IgGλ BP-PE: sc-516186 (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 DAGLβ gene expression knockdown using RT-PCR Primer: DAGLβ (h)-PR: sc-89591-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.