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SANTA CRUZ BIOTECHNOLOGY, INC.

DGK-θ siRNA (h): sc-45681

BACKGROUND

Diacylglycerol kinases (DGKs) phosphorylate diacylglycerol (DAG) to produce phosphatidic acid. DAG and phosphatidic acid are lipids that act as second messengers in signaling cascades. DGK-α influences cell activation and secretion of lethal exosomes, which in turn control cell death. DGK-β is abundant in restricted brain regions such as the caudate putamen and olfactory tubercle. DGK-γ encodes full-length and truncated transcripts that are present in a range of human tissues, with greatest expression observed in retina. DGK-δ is most abundant in skeletal muscle. DGK-ε shows specificity for arachidonyl-containing diacylglycerol and is expressed predominantly in testis. DGK-ζ is most abundant in brain and muscle. DGK-η is closely related to DGK-δ. DGK-θ is most abundant in the cerebellum and hippocampus. DGK-ι is present in brain and retina as a predominant transcript of more than 12 kb, including a long 3' untranslated region, with additional low abundance transcripts of 9.5 and 7.5 kb. DGKs have structural motifs that play regulatory roles, and these motifs form the basis for dividing the DGKs into five subtypes.

REFERENCES

1. Schaap, D., et al. 1990. Purification, cDNA-cloning and expression of human diacylglycerol kinase. FEBS Lett. 275: 151-158.
2. Goto, K., et al. 1993. Molecular cloning and expression of a 90 kDa diacylglycerol kinase that predominantly localizes in neurons. Proc. Natl. Acad. Sci. USA 90: 7598-7602.
3. Kai, M., et al. 1994. Molecular cloning of a diacylglycerol kinase isozyme predominantly expressed in human retina with a truncated and inactive enzyme expression in most other human cells. J. Biol. Chem. 269: 18492-18498.
4. Sakane, F., et al. 1996. Molecular cloning of a novel diacylglycerol kinase isozyme with a pleckstrin homology domain and a C-terminal tail similar to those of the EPH family of protein-tyrosine kinases. J. Biol. Chem. 271: 8394-8401.

CHROMOSOMAL LOCATION

Genetic locus: DGKQ (human) mapping to 4p16.3.

PRODUCT

DGK-θ 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 DGK-θ shRNA Plasmid (h): sc-45681-SH and DGK-θ shRNA (h) Lentiviral Particles: sc-45681-V as alternate gene silencing products.

For independent verification of DGK-θ (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-45681A, sc-45681B and sc-45681C.

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

DGK-θ siRNA (h) is recommended for the inhibition of DGK-θ 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

DGK-θ (E-5): sc-137197 is recommended as a control antibody for monitoring of DGK-θ 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 DGK-θ gene expression knockdown using RT-PCR Primer: DGK-θ (h)-PR: sc-45681-PR (20 µl, 399 bp). 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.