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DGK- θ shRNA (h) Lentiviral Particles: sc-45681-V

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-prime 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.

CHROMOSOMAL LOCATION

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

PRODUCT

DGK- θ shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see DGK- θ siRNA (h): sc-45681 and DGK- θ shRNA Plasmid (h): sc-45681-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

DGK- θ shRNA (h) Lentiviral Particles is recommended for the inhibition of DGK- θ expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker[™] compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

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 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.