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PI 4-kinase α shRNA (h) Lentiviral Particles: sc-44012-V

BACKGROUND

The members of the phosphatidylinositol kinase (PIK) superfamily can be divided into three groups based on their substrate specificity. PIKs convert phosphatidylinositol (PI) into PI phosphate [PI(3)P], PI phosphate [PI(4)P], PI bisphosphate [PI(4,5)P₂] and PI triphosphate [PI(3,4,5)P₃]. The first group, the PI 3-kinases, is composed of highly related proteins designated p110 α , p110 β , p110 γ and p110 δ which convert PI into PI(3)P and PI(4,5)P₂ into PI(3,4,5)P₃. The second group, the PI 4-kinases, convert PI into PI(4)P. The third group, the PI(4)P5-kinases, convert PI(4)P into PI(4,5)P₂. Phosphatidylinositides represent important regulatory molecules and are involved in a diverse array of signaling pathways. Phosphatidylinositol biphosphate acts as an activator of PKCs and as a substrate for PLC γ , which converts the molecule into the second messengers, inositol-1,4,5 triphosphate and 1,2-diacylglycerol. PI(3,4,5)P₃ has been shown to activate the PKC ζ isoform. Wortmannin, originally described as a specific inhibitor of PI 3-kinases, may actually be a broad spectrum inhibitor of PI kinase activity.

REFERENCES

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CHROMOSOMAL LOCATION

Genetic locus: PI4KA (human) mapping to 22q11.21.

PRODUCT

PI 4-kinase α 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 PI 4-kinase α siRNA (h): sc-44012 and PI 4-kinase α shRNA Plasmid (h): sc-44012-SH as alternate gene silencing products.

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.

APPLICATIONS

PI 4-kinase α shRNA (h) Lentiviral Particles is recommended for the inhibition of PI 4-kinase α 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

PI 4-kinase α (H-142): sc-10799 is recommended as a control antibody for monitoring of PI 4-kinase α 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PI 4-kinase α gene expression knockdown using RT-PCR Primer: PI 4-kinase α (h)-PR: sc-44012-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.

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.

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