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SANTA CRUZ BIOTECHNOLOGY, INC.

PLC β3 siRNA (h2): sc-44321

BACKGROUND

Phosphoinositide-specific phospholipase C (PLC) plays a critical role in the initiation of receptor mediated signal transduction through the generation of the two second messengers, inositol 1, 4, 5-triphosphate and diacylglycerol from phosphatidylinositol 4, 5 bisphosphate. A total of eight mammalian PLC isozymes have been described (PLC β1, PLC β2, PLC β3, PLC β4, PLC γ1, PLC γ2, PLC δ1 and PLC δ2). The γ-type enzymes are unique in that they contain SH2 and SH3 domains. Moreover, the two γ-type enzymes, but not the β and δ isozymes, are subject to activation by a number of protein tyrosine kinases which associate with their SH2 domains and induce their activation by phosphoryation. In contrast, activation of PLC β1, PLC β2 and PLC β3 is mediated by the a subunits of the G_q class of heterotrimeric G proteins and by certain βγ G protein subunits. The regulatory mechanisms for PLC δ1 and PLC δ2 are as yet not resolved.

REFERENCES

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2. Emori, Y., et al. 1989. A second type of rat phosphoinositide-specific phospholipase C containing a Src-related sequence not essential for phosphoinositide-hydrolyzing activity. J. Biol. Chem. 264: 21885-21890.
3. Meldrum, E., et al. 1991. A second gene product of the inositol-phospholipid-specific phospholipase Cδ subclass. Eur. J. Biochem. 196: 159-165.
4. Koch, C.A., et al. 1991. SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. Science 252: 668-674.
5. Rhee, S.G. and Choi, K.D. 1992. Regulation of inositol phospholipid-specific phospholipase C isozymes. J. Biol. Chem. 267: 12393-12396.
6. Jhon, D., et al. 1993. Cloning, sequencing, purification and G_q-dependent activation of phospholipase C-β3. J. Biol. Chem. 268: 6654-6661.

CHROMOSOMAL LOCATION

Genetic locus: Plcb3 (mouse) mapping to 19 A.

PRODUCT

PLC β3 siRNA (h2) 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 PLC β3 shRNA Plasmid (h2): sc-44321-SH and PLC β3 shRNA (h2) Lentiviral Particles: sc-44321-V as alternate gene silencing products.

For independent verification of PLC β3 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-44321A, sc-44321B and sc-44321C.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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

PLC β3 siRNA (h2) is recommended for the inhibition of PLC β3 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

PLC β3 (H-1): sc-271372 is recommended as a control antibody for monitoring of 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 gene expression knockdown using RT-PCR Primer: PLC β3 (h2)-PR: sc-44321-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.