

PLC β 3 siRNA (r): sc-156124

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 biphosphate. A total of eight mammalian PLC isozymes have been described (PLC β 1, PLC β 2, PLC β 3, PLC β 4, PLC γ 1, PLC γ 2, PLC δ 1 and PLC δ 2). 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 phosphorylation. In contrast, activation of PLC β 1, PLC β 2 and PLC β 3 is mediated by the α subunits of the G_q class of heterotrimeric G proteins and by certain $\beta\gamma$ protein subunits. The regulatory mechanisms for PLC δ 1 and PLC δ 2 are not yet resolved.

REFERENCES

1. Suh, P., et al. 1988. Inositol phospholipid-specific phospholipase C: complete cDNA and protein sequences and sequence homology to tyrosine kinase-related oncogene products. *Proc. Natl. Acad. Sci. USA* 85: 5419-5423.
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. Koch, C.A., et al. 1991. SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. *Science* 252: 668-674.
4. Meldrum, E., et al. 1991. A second gene product of the inositol-phospholipid-specific phospholipase C subclass. *Eur. J. Biochem.* 196: 159-165.
5. Rhee, S.G. and Choi, K.D. 1992. Regulation of inositol phospholipid-specific phospholipase C isozymes. *J. Biol. Chem.* 267: 12393-12396.
6. Kim, M.J., et al. 1993. Cloning of cDNA encoding rat phospholipase C- β 4, a new member of the phospholipase C. *Biochem. Biophys. Res. Comm.* 194: 706-712.
7. Jhon, D., et al. 1993. Cloning, sequencing, purification and G_q -dependent activation of phospholipase C- β 3. *J. Biol. Chem.* 268: 6654-6661.

CHROMOSOMAL LOCATION

Genetic locus: Plcb3 (rat) mapping to 1q43.

PRODUCT

PLC β 3 siRNA (r) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PLC β 3 shRNA Plasmid (r): sc-156124-SH and PLC β 3 shRNA (r) Lentiviral Particles: sc-156124-V as alternate gene silencing products.

For independent verification of PLC β 3 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-156124A, sc-156124B and sc-156124C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PLC β 3 siRNA (r) is recommended for the inhibition of PLC β 3 expression in rat 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 μ M in 66 μ l. 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 β 3 (D-7): sc-133231 is recommended as a control antibody for monitoring of PLC β 3 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 PLC β 3 gene expression knockdown using RT-PCR Primer: PLC β 3 (r)-PR: sc-156124-PR (20 μ l). 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.

PROTOCOLS

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