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SANTA CRUZ BIOTECHNOLOGY, INC.

Pleckstrin siRNA (m): sc-152303

BACKGROUND

Activation of protein kinase C (PKC) in platelets results in immediate phosphorylation of Pleckstrin (previously called 40K or P47), the major PKC substrate in platelets. Pleckstrin contains a Ca^{2+}-binding "EF-hand" structure and PKC phosphorylation sites at Ser 113 and Ser 117. The N and C termini of Pleckstrin contain two Pleckstrin homology domains (PH), which mediate protein-protein and protein-lipid interactions. Pleckstrin is highly expressed in human neutrophils. Pleckstrin is rapidly phosphorylated following treatment of neutrophils in response to inflammatory stimuli, probably by nonconventional PKC isoforms δ or ζ, which are expressed in human neutrophils. Phosphorylation by nonconventional PKC isoforms induces a conformational change in Pleckstrin that promotes its interaction with membranes and/or with the cytoskeleton, serving to target proteins or lipids recognized by PH domains to sites where they can contribute to the microbicidal response.

REFERENCES

1. Tyers, M., et al. 1988. Molecular cloning and expression of the major protein kinase C substrate of platelets. Nature 333: 470-473.
2. Tyers, M., et al. 1989. Molecular analysis of Pleckstrin: the major protein kinase C substrate of platelets. J. Cell. Biochem. 40: 133-145.
3. Yoon, H.S., et al. 1994. Solution structure of a Pleckstrin-homology domain. Nature 369: 672-675.
4. Abrams, C.S., et al. 1995. Protein kinase C regulates pleckstrin by phosphorylation of sites adjacent to the N-terminal pleckstrin homology domain. J. Biol. Chem. 270: 23317-23321.
5. Craig, K.L. and Harley, C.B. 1996. Phosphorylation of human pleckstrin on Ser-113 and Ser-117 by protein kinase C. Biochem. J. 314: 937-942.
6. Brumell, J.H., et al. 1997. Phosphorylation and subcellular redistribution of Pleckstrin in human neutrophils. J. Immunol. 158: 4862-4871.
7. Cmarik, J.L., et al. 2000. cDNA cloning and mapping of mouse Pleckstrin (Plek), a gene upregulated in transformation-resistant cells. Genomics 66: 204-212.

CHROMOSOMAL LOCATION

Genetic locus: Plek (mouse) mapping to 11 A2.

PRODUCT

Pleckstrin siRNA (m) 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 Pleckstrin shRNA Plasmid (m): sc-152303-SH and Pleckstrin shRNA (m) Lentiviral Particles: sc-152303-V as alternate gene silencing products.

For independent verification of Pleckstrin (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-152303A, sc-152303B and sc-152303C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

Pleckstrin siRNA (m) is recommended for the inhibition of Pleckstrin expression in mouse 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

Pleckstrin (25): sc-136042 is recommended as a control antibody for monitoring of Pleckstrin 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 Pleckstrin gene expression knockdown using RT-PCR Primer: Pleckstrin (m)-PR: sc-152303-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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