

PYK2 siRNA (m): sc-36333

BACKGROUND

Focal adhesion kinase (FAK) was initially identified as a substrate for the intrinsic protein tyrosine kinase activity of Src-encoded pp60. The deduced amino acid sequence of FAK p125 has shown it to be a cytoplasmic protein tyrosine kinase whose sequence and structural organization are unique compared to other protein families described. A putative new member of the FAK family, designated PYK2 (proline-rich tyrosine kinase 2), exhibits 61% sequence identity with FAK over its kinase domain. PYK2 (also designated CAK β or RAFTK) is highly expressed in the central nervous system. Activation of the kinase leads to modulation of ion channel function and the activation of the MAPK signaling pathway. PYK2 is rapidly phosphorylated on tyrosine residues in response to stimuli that increase intracellular calcium levels and compounds that activate members of the PKC family of kinases, such as phorbol esters.

REFERENCES

- Schaller, M.D., et al. 1992. FAK p125, a structurally distinctive protein-tyrosine kinase associated with focal adhesions. *Proc. Natl. Acad. Sci. USA* 89: 5192-5196.
- Lipfert, L., et al. 1992. Integrin-dependent phosphorylation of the protein tyrosine kinase FAK p125 in platelets. *J. Cell. Biol.* 119: 905-912.
- Hanks, S.K., et al. 1992. Focal adhesion protein-tyrosine kinase phosphorylated in response to cell attachment to fibronectin. *Proc. Natl. Acad. Sci. USA* 89: 8487-8491.
- Guan, J.L., et al. 1992. Regulation of focal adhesion-associated protein tyrosine kinase by both cellular adhesion and oncogenic transformation. *Nature* 359: 690-692.
- Schaller, M.D., et al. 1994. Autophosphorylation of the focal adhesion-associated protein tyrosine kinase, pp125FAK, directs SH2-dependent binding of pp60Src. *Mol. Cell. Biol.* 14: 1680-1688.
- Lev, S., et al. 1995. Protein tyrosine kinase PYK2 involved in Ca²⁺-induced regulation of ion channel and MAP kinase functions. *Nature* 376: 737-745.

CHROMOSOMAL LOCATION

Genetic locus: Ptk2b (mouse) mapping to 14 D1.

PRODUCT

PYK2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PYK2 shRNA Plasmid (m): sc-36333-SH and PYK2 shRNA (m) Lentiviral Particles: sc-36333-V as alternate gene silencing products.

For independent verification of PYK2 (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-36333A, sc-36333B and sc-36333C.

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 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

PYK2 siRNA (m) is recommended for the inhibition of PYK2 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 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

PYK2 (E-3): sc-393181 is recommended as a control antibody for monitoring of PYK2 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 PYK2 gene expression knockdown using RT-PCR Primer: PYK2 (m)-PR: sc-36333-PR (20 μ l, 428 bp). 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.