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FAK siRNA (r): sc-156037

BACKGROUND

Focal adhesion kinase was initially identified as a major 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 as compared to other proteins described to date. Localization of p125 by immunofluorescence suggests that it is primarily found in cellular focal adhesions leading to its designation as focal adhesion kinase (FAK). FAK is concentrated at the basal edge of only those basal keratinocytes that are actively migrating and rapidly proliferating in repairing burn wounds and is activated and localized to the focal adhesions of spreading keratinocytes in culture. Thus, it has been postulated that FAK may have an important *in vivo* role in the reepithelialization of human wounds. FAK protein tyrosine kinase activity has also been shown to increase in cells stimulated to grow by use of mitogenic neuropeptides or neurotransmitters acting through G protein-coupled receptors.

REFERENCES

- Schaller, M.D., et al. 1992. pp125^{FAK}, a structurally distinctive protein-tyrosine kinase associated with focal adhesions. *Proc. Natl. Acad. Sci. USA* 89: 5192-5196.
- 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.

CHROMOSOMAL LOCATION

Genetic locus: Ptk2 (rat) mapping to 7q34.

PRODUCT

FAK 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 FAK shRNA Plasmid (r): sc-156037-SH and FAK shRNA (r) Lentiviral Particles: sc-156037-V as alternate gene silencing products.

For independent verification of FAK (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-156037A, sc-156037B and sc-156037C.

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

FAK siRNA (r) is recommended for the inhibition of FAK 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

FAK (D-1): sc-271126 is recommended as a control antibody for monitoring of FAK 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 FAK gene expression knockdown using RT-PCR Primer: FAK (r)-PR: sc-156037-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Wang, X., et al. 2012. Focal adhesion kinase activates NF κ B via the ERK 1/2 and p38MAPK pathways in Amyloid- β ₂₅₋₃₅-induced apoptosis in PC12 cells. *J. Alzheimers Dis.* 32: 77-94.
- Cheng, Z., et al. 2017. Focal adhesion kinase-mediated phosphorylation of beclin1 protein suppresses cardiomyocyte autophagy and initiates hypertrophic growth. *J. Biol. Chem.* 292: 2065-2079.

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.