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FAK shRNA (h2) Lentiviral Particles: sc-44207-V

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.
- Lipfert, L., et al. 1992. Integrin-dependent phosphorylation of the protein tyrosine kinase pp125 FAK in platelets. *J. Cell Biol.* 119: 905-912.
- 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 (human) mapping to 8q24.3.

PRODUCT

FAK shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see FAK siRNA (h2): sc-44207 and FAK shRNA Plasmid (h2): sc-44207-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

FAK shRNA (h2) Lentiviral Particles is recommended for the inhibition of FAK expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

FAK (B-8): sc-271195 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 FAK gene expression knockdown using RT-PCR Primer: FAK (h2)-PR: sc-44207-PR (20 μ l, 515 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

SELECT PRODUCT CITATIONS

- Hamidouche, Z., et al. 2009. Priming integrin α 5 promotes human mesenchymal stromal cell osteoblast differentiation and osteogenesis. *Proc. Natl. Acad. Sci. USA* 106: 18587-18591.
- Hamidouche, Z., et al. 2010. Crosstalks between integrin α 5 and IGF2/IGFBP2 signalling trigger human bone marrow-derived mesenchymal stromal osteogenic differentiation. *BMC Cell Biol.* 11: 44.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.