SANTA CRUZ BIOTECHNOLOGY, INC.

FAPα siRNA (h): sc-62292

BACKGROUND

FAPα, or seprase, is a 760 amino acid protein encoded by the human gene FAP and belongs to the peptidase S9B family. FAPα may have a role in tissue remodeling during development and wound healing so it is possible FAPα may contribute to invasiveness of malignant cancers. It degrades gelatin and heat-denatured type I and type IV collagen, but not native type I or type IV collagen. It also does not cleave Laminin, Fibronectin, fibrin or casein. FAPα is a single-pass type II membrane protein found on cell surface lamellipodia, invadopodia and on shed vesicles. FAPα is usually found as a glycosylated homodimer, or heterodimer with DPP4. The FAPα monomer is an inactive form.

REFERENCES

1. Aertgeerts, K., et al. 2005. Structural and kinetic analysis of the substrate specificity of human fibroblast activation protein α. J. Biol. Chem. 280: 19441-19444.
2. Kelly, T. 2005. Fibroblast activation protein α and dipeptidyl peptidase IV (CD26): cell-surface proteases that activate cell signaling and are potential targets for cancer therapy. Drug Resist. Updat. 8: 51-58.
3. Dolznig, H., et al. 2005. Characterization of cancer stroma markers: in silico analysis of an mRNA expression database for fibroblast activation protein and endosialin. Cancer Immun. 5: 10-10.
4. Iwasa, S., et al. 2005. Increased expression of seprase, a membrane-type serine protease, is associated with lymph node metastasis in human colo-rectal cancer. Cancer Lett. 227: 229-236.
5. Terret, M.E., et al. 2006. Meiosis: separase strikes twice. Nat. Cell Biol. 8: 910-911.

CHROMOSOMAL LOCATION

Genetic locus: FAP (human) mapping to 2q24.2.

PRODUCT

FAPα siRNA (h) 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 FAPα shRNA Plasmid (h): sc-62292-SH and FAPα shRNA (h) Lentiviral Particles: sc-62292-V as alternate gene silencing products.

For independent verification of FAPα (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-62292A, sc-62292B and sc-62292C.

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

FAPα siRNA (h) is recommended for the inhibition of FAPα expression in human 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

FAPα (SS-13): sc-100528 is recommended as a control antibody for monitoring of FAPα 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 FAPα gene expression knockdown using RT-PCR Primer: FAPα (h)-PR: sc-62292-PR (20 µl, 573 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Higashino, N., et al. 2019. Fibroblast activation protein-positive fibroblasts promote tumor progression through secretion of CCL2 and interleukin-6 in esophageal squamous cell carcinoma. Lab. Invest. 99: 777-792.

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.