SANTA CRUZ BIOTECHNOLOGY, INC.

PFAAP5 siRNA (h): sc-76110

BACKGROUND

PFAAP5 (phosphonoformate immuno-associated protein 5), also known as NEDD4-binding protein 2-like 2, is a 583 amino acid nuclear protein that potentially is involved in transcriptional regulation. PFAAP5 is phosphorylated on Ser 199 in response to DNA damage, probably by ATM or ATR. Primarily expressed in bone marrow, PFAAP5 is dramatically down-regulated after exposure to arsenic compounds, an event which precedes neutropenia. PFAAP5 interacts with both Gfi-1 and Neutrophil Elastase, two proteins that are implicated in neutropenia disorders. Defects in the gene encoding Neutrophil Elastase, ELA2, are the cause of cyclic haematopoiesis, which, with decreased numbers of circulating neutrophils, leads to an increased risk for opportunistic infection. Gfi1 is a transcriptional repressor that targets the ELA2 gene among others. With PFAAP5 expression, Neutrophil Elastase can potentiate repression of Gfi1 target genes, leading to the transcription of ELA2 and subsequent neutrophil differentiation. There are two isoforms of PFAAP5 that are produced as a result of alternative splicing events.

REFERENCES

1. Couch, F.J., et al. 1996. Generation of an integrated transcription map of the BRCA2 region on chromosome 13q12-q13. Genomics 36: 86-99.
2. Grimes, H.L., et al. 1996. The Gfi-1 protooncoprotein represses Bax expression and inhibits T-cell death. Proc. Natl. Acad. Sci. USA 93: 14569-14573.
3. Doan, L.L., et al. 2004. Targeted transcriptional repression of Gfi1 by GFI1 and GFI1B in lymphoid cells. Nucleic Acids Res. 32: 2508-2519.
4. Argos, M., et al. 2006. Gene expression profiles in peripheral lymphocytes by arsenic exposure and skin lesion status in a Bangladeshi population. Cancer Epidemiol. Biomarkers Prev. 15: 1367-1375.
5. Matsuoka, S., et al. 2007. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science 316: 1160-1166.
6. Zeidler, C., et al. 2009. Clinical implications of ELA2-, HAX1-, and G-CSF-receptor (CSF3R) mutations in severe congenital neutropenia. Br. J. Haematol. 144: 459-467.

CHROMOSOMAL LOCATION

Genetic locus: N4BP2L2 (human) mapping to 13q13.1.

PRODUCT

PFAAP5 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 PFAAP5 shRNA Plasmid (h): sc-76110-SH and PFAAP5 shRNA (h) Lentiviral Particles: sc-76110-V as alternate gene silencing products.

For independent verification of PFAAP5 (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-76110A, sc-76110B and sc-76110C.

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

PFAAP5 siRNA (h) is recommended for the inhibition of PFAAP5 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

PFAAP5 (F-2): sc-514596 is recommended as a control antibody for monitoring of PFAAP5 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 PFAAP5 gene expression knockdown using RT-PCR Primer: PFAAP5 (h)-PR: sc-76110-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.