SANTA CRUZ BIOTECHNOLOGY, INC.

PAPP-A siRNA (h): sc-61289

BACKGROUND

Pregnancy-associated plasma protein-A (Pappalysin-1 or PAPP-A), also known as Insulin-like growth factor-dependent IGF-binding protein 4 (IGFBP4) protease, is a member of the peptidase M43B family of proteins. PAPP-A, a metalloproteinase, cleaves Insulin-like growth factor binding proteins IGFBP4 and IGFBP5, releasing bound IGF. PAPP-A is primarily expressed in septa and anchoring villi in placenta and is also expressed in pregnancy serum. Levels of PAPP-A increase throughout pregnancy. Lower levels of expression can be detected in kidney, prostate, breast, ovary and endometrial tissues. PAPP-A is a secreted protein that can form homodimers; in pregnancy serum PAPP-A may also form a heterotetramer with PRG-2.

REFERENCES

1. Kristensen, T., et al. 1994. Amino acid from cloned cDNA. Biochemistry 33: 1592-1598.
2. Fortune, J.E., et al. 2004. Follicular development: the role of the follicula of the dominant follicle. Anim. Reprod. Sci. 82-83: 109-126.
3. Bunn, R.C., et al. 2004. IGFBP-4 degradation by pregnancy-associated plasma protein-A in MC3T3 osteoblasts. Biochem. Biophys. Res. Commun. 325: 698-706.
4. Kalli, K.R., et al. 2004. Pregnancy-associated plasma protein-A (PAPP-A) expression and Insulin-like growth factor binding protein-4 protease activity in normal and malignant ovarian surface epithelial cells. Int. J. Cancer 110: 633-640.
5. Spicer, L.J. 2004. Proteolytic degradation of Insulin-like growth factor binding proteins by ovar follicles: a control mechanism for selection of dominant follicles. Biol. Reprod. 70: 1223-1230.
6. Santolaya-Forgas, J., et al. 2004. Low pregnancy-associated plasma protein-A at 10^{+1} to 14^{+6} weeks of gestatio and a possible mechanism leading to miscarriage. Fetal Diagn. Ther. 19: 456-461.
7. Santiago, C.A., et al. 2004. protein mRNAs in granulosa cells of dominant and subordinate follicles of preovulatory cattle. Domest. Anim. Endocrinol. 28: 46-63.
8. García, J., et al. 2005. Identification of two novel human genes, DIPLA1 and DIPAS, tissue. Gene 344: 241-250.

CHROMOSOMAL LOCATION

Genetic locus: PAPPA (human) mapping to 9q33.1.

PRODUCT

PAPP-A 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 PAPP-A shRNA Plasmid (h): sc-61289-SH and PAPP-A shRNA (h) Lentiviral Particles: sc-61289-V as alternate gene silencing products.

For independent verification of PAPP-A (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-61289A, sc-61289B and sc-61289C.

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

PAPP-A siRNA (h) is recommended for the inhibition of PAPP-A 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

PAPP-A (B-7): sc-365226 is recommended as a control antibody for monitoring of PAPP-A 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 PAPP-A gene expression knockdown using RT-PCR Primer: PAPP-A (h)-PR: sc-61289-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.