SANTA CRUZ BIOTECHNOLOGY, INC.

WAP siRNA (m): sc-37182

BACKGROUND

Whey acidic protein (WAP), a hormonally-regulated acidic, cysteine-rich protein, is a major whey protein found in rodent milk and may belong to a family of protease inhibitors. The WAP gene consists of four exons and three introns. The middle two exons encode the two cysteine-rich regions which form separate protein domains. WAP also contain a N-terminal signal peptide of 19 amino acids. The WAP gene is expressed in mammary epithelial cells, is induced several thousand-fold during pregnancy and is under the control of lactogenic hormones. Induction of WAP gene is caused by glucocorticoid, PRL, and Insulin. Expression of WAP mRNA is highly dependent on stage of estrous, with detection restricted to midcycle. Low levels of WAP RNA are found in some nonmammary tissues such as tongue, pancreas, and pituitary gland, but not in others, for examle, heart and brain. WAP secretion in milk occurs throughout lactation and is restricted to number of species, including mouse, rat, rabbit, camel, and porcine. Mouse mammary epithelial cells cultured on basement membrane-type matrix express high levels of WAP mRNA and secrete the protein into the lumen. This expression is dependent upon the formation of the alveoli-like spheres.

REFERENCES

1. Hennighausen, L.G. and Sippe, A.E. 1982. Mouse whey acidic protein is a novel member of the family of "four-disulfide core" proteins. Nucleic Acids Res. 10: 2677-2684.
2. Hennighausen, L.G., et al. 1982. Comparative sequence analysis of the mRNAs coding for mouse and rat whey protein. Nucleic Acids Res. 10: 3733-3744.
3. Campbell, S.M., et al. 1984. Comparison of the whey acidic protein genes of the rat and mouse. Nucleic Acids Res. 12: 8685-8697.
4. Andres, A.C., et al. 1987. Ha-Ras oncogene expression directed by a milk protein gene promoter: tissue specificity, hormonal regulation, and tumor induction in transgenic mice. Proc. Natl. Acad. Sci. USA 84: 1299-1303.
5. Doppler, W., et al. 1991. Lactogenic hormone and cell type-specific control of the whey acidic protein gene promoter in transfected mouse cells. Mol. Endocrinol. 5: 1624-1632.
6. Hennighausen, L., et al. 1991. Regulation of expression of genes for milk proteins. Biotechnology 16: 65-74.

CHROMOSOMAL LOCATION

Genetic locus: Wap (mouse) mapping to 11 A1.

PRODUCT

WAP siRNA (m) 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 WAP shRNA Plasmid (m): sc-37182-SH and WAP shRNA (m) Lentiviral Particles: sc-37182-V as alternate gene silencing products.

For independent verification of WAP (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-37182A, sc-37182B and sc-37182C.

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

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

WAP (E-8): sc-398276 is recommended as a control antibody for monitoring of WAP 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 WAP gene expression knockdown using RT-PCR Primer: WAP (m)-PR: sc-37182-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.