

β-casein siRNA (h): sc-40384

BACKGROUND

Milk proteins are crucial for the development of all newborn mammals and caseins constitute the major proteins in mammalian milk. β- and κ-caseins are the only caseins present in human milk. The β-casein/κ-casein ratio is higher in colostrum than in transitional and mature milk and is related to a better digestibility of colostrum casein micelles by the neonate during the first days of life. Human β-casein-encoding gene (Bca) contains a highly phosphorylated site, which is responsible for the calcium-binding capacity of β-casein. A common set of transcription factors are required for the expression of β-casein. Multiple binding sites for Stat5, C/EBPβ (CCAAT/enhancer-binding protein) and several half-sites for glucocorticoid receptor (GR) are identified in the distal human enhancer of the β-casein gene. β-casein gene transcription is regulated primarily by a composite response element (CoRE), which integrates signaling from the lactogenic hormones PRL, Insulin and hydrocortisone in mammary epithelial cells. NFκB functions as a negative regulator of β-casein gene expression during pregnancy by interfering with Stat5 tyrosine phosphorylation.

REFERENCES

1. Greenberg, R., et al. 1984. Human β-casein. Amino acid sequence and identification of phosphorylation sites. *J. Biol. Chem.* 259: 5132-5138.
2. Lonnerdal, B., et al. 1990. Cloning and sequencing of a cDNA encoding human milk β-casein. *FEBS Letts.* 269: 153-156.
3. Menon, R.S., et al. 1992. Regional localization of human β-casein gene (CSN2) to 4pter-q21. *Genomics* 13: 25-26.
4. Hansson, L., et al. 1994. Structure of the human β-casein encoding gene. *Gene* 139: 193-199.
5. Winklehner-Jennewein, P., et al. 1998. A distal enhancer region in the human β-casein gene mediates the response to prolactin and glucocorticoid hormones. *Gene* 217: 127-139.
6. Cuilliere, M.L., et al. 1999. Changes in the κ-casein and β-casein concentrations in human milk during lactation. *J. Clin. Lab. Anal.* 13: 213-218.
7. Lykos, M.A., et al. 2000. Autocrine insulin-like growth factor II inhibits β-casein mRNA expression in a mammary cell line. *J. Dairy Sci.* 83: 285-295.

CHROMOSOMAL LOCATION

Genetic locus: CSN2 (human) mapping to 4q13.3.

PRODUCT

β-casein 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 β-casein shRNA Plasmid (h): sc-40384-SH and β-casein shRNA (h) Lentiviral Particles: sc-40384-V as alternate gene silencing products.

For independent verification of β-casein (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-40384A, sc-40384B and sc-40384C.

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

β-casein siRNA (h) is recommended for the inhibition of β-casein 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

β-casein (F20.14): sc-53189 is recommended as a control antibody for monitoring of β-casein 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 β-casein gene expression knockdown using RT-PCR Primer: β-casein (h)-PR: sc-40384-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.