SANTA CRUZ BIOTECHNOLOGY, INC.

A33 siRNA (m): sc-44822

BACKGROUND

The A33 antigen is a transmembrane protein expressed almost exclusively in intestinal epithelium and in 95% of human colon cancers. Specifically, expression of the A33 protein occurs on the basolateral surfaces of intestinal epithelial cells of all lineages. The A33 antigen comprises an extracellular domain with two immunoglobulin-like domains, a single-span transmembrane domain, and a highly acidic cytoplasmic domain. Expression of A33 appears to be regulated by the intestine-specific homeobox transcription factor CDX1 and the gut-enriched Krüppel-like factor (GKLF). GKLF binds to the promoter region of the A33 gene in colon cancer cells, and mutations in the GKLF binding sequence leads to reduced expression of the A33 antigen. The therapeutic potential of administering the humanized monoclonal antibody A33 to colon cancer patients has proved encouraging.

REFERENCES

1. Ritter, G., et al. 1997. Characterization of posttranslational modifications of human A33 antigen, a novel palmitoylated surface glycoprotein of human gastrointestinal epithelium. Biochem. Biophys. Res. Commun. 236: 682-686.
2. Heath, J.K., et al.1997. The human A33 antigen is a transmembrane glycoprotein and a novel member of the immunoglobulin superfamily. Proc. Natl. Acad. Sci. USA 94: 469-474.
3. Abud, H.E., et al. 2000. The murine A33 antigen is expressed at two distinct sites during development, the ICM of the blastocyst and the intestinal epithelium. Mech. Dev. 98:111-114.
4. Johnstone, C.N., et al. 2000. Characterization of mouse A33 antigen, a definitive marker for basolateral surfaces of intestinal epithelial cells. 2000. Am. J. Physiol. Gastrointest. Liver Physiol. 279: G500-G510.
5. Sakamoto, J., et al. 2000. Organ-specific expression of the intestinal epithelium-related antigen A33, a cell surface target for antibody-based imaging and treatment in gastrointestinal cancer. Cancer Chemother Pharmacol. 46: S27-S32.
6. Johnstone, C.N., et al. 2002. Analysis of the regulation of the A33 antigen gene reveals intestine-specific mechanisms of gene expression. J. Biol. Chem. 277: 34531-34539.

CHROMOSOMAL LOCATION

Genetic locus: Gpa33 (mouse) mapping to 1 H2.3.

PRODUCT

A33 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 A33 shRNA Plasmid (m): sc-44822-SH and A33 shRNA (m) Lentiviral Particles: sc-44822-V as alternate gene silencing products.

For independent verification of A33 (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-44822A, sc-44822B and sc-44822C.

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

A33 siRNA (m) is recommended for the inhibition of A33 expression in mouse 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

A33 (E-8): sc-398702 is recommended as a control antibody for monitoring of A33 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 A33 gene expression knockdown using RT-PCR Primer: A33 (m)-PR: sc-44822-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.