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A33 shRNA (h) Lentiviral Particles: sc-44821-V

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 Kruppel-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

- 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.
- 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.
- 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.
- 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.

CHROMOSOMAL LOCATION

Genetic locus: GPA33 (human) mapping to 1q24.1.

PRODUCT

A33 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see A33 siRNA (h): sc-44821 and A33 shRNA Plasmid (h): sc-44821-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

A33 shRNA (h) Lentiviral Particles is recommended for the inhibition of A33 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

A33 (FL-319): sc-50522 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor A33 gene expression knockdown using RT-PCR Primer: A33 (h)-PR: sc-44821-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.