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RBP shRNA (h) Lentiviral Particles: sc-44071-V

BACKGROUND

Retinol (Vitamin A) is transported in the blood bound to its carrier protein, retinol-binding protein (RBP), also designated plasma retinol-binding protein (PRBP) or RBP4. A member of the lipocalin family, RBP conveys retinol from stores in the liver to peripheral tissues. In plasma, RBP binds transthyretin (TTR, formerly called prealbumin) to prevent glomerular filtration of low molecular weight RBP in the kidneys. The stability of this complex holds diagnostic importance because the molar ratio of RBP:TTR provides an indirect way to indicate marginal vitamin A deficiency. Vitamin A deficiency blocks the secretion of RBP resulting in defective delivery and supply to epidermal cells. Originally identified solely as a transporter protein, recent studies correlating increased levels of RBP expression in adipose tissue with Insulin resistance have generated research into the possible roles the protein may play in the pathogenesis of type 2 diabetes and obesity.

REFERENCES

1. van Bennekum, A.M., et al. 1993. Retinol uptake from retinol-binding protein (RBP) by liver parenchymal cells *in vitro* does not specifically depend on its binding to RBP. *Biochemistry* 32: 1727-1733.
2. Zanotti, G., et al. 1993. The interaction of N-ethyl retinamide with plasma retinol-binding protein (RBP) and the crystal structure of the retinoid-RBP complex at 1.9-Å resolution. *J. Biol. Chem.* 268: 24873-24879.
3. Yamamoto, Y., et al. 1997. Interactions of transthyretin (TTR) and retinol-binding protein (RBP) in the uptake of retinol by primary rat hepatocytes. *Exp. Cell. Res.* 234: 373-378.
4. Naylor, H.M., et al. 1999. The structure of human retinol-binding protein (RBP) with its carrier protein transthyretin reveals an interaction with the carboxy terminus of RBP. *Biochemistry* 38: 2647-2653.
5. Quadro, L., et al. 2002. Muscle expression of human retinol-binding protein (RBP). Suppression of the visual defect of RBP knockout mice. *J. Biol. Chem.* 277: 30191-30197.

CHROMOSOMAL LOCATION

Genetic locus: RBP4 (human) mapping to 10q23.33.

PRODUCT

RBP 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 µl frozen stock containing 1.0 x 10⁶ 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 RBP siRNA (h): sc-44071 and RBP shRNA Plasmid (h): sc-44071-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.

APPLICATIONS

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

RBP (C-4): sc-48384 is recommended as a control antibody for monitoring of RBP 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor RBP gene expression knockdown using RT-PCR Primer: RBP (h)-PR: sc-44071-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

PROTOCOLS

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