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FcRn shRNA (h) Lentiviral Particles: sc-45632-V

BACKGROUND

FcRn present in the intestinal epithelium of neonatal mice and rats mediates the selective uptake of immunoglobulin G (IgG) in mothers' milk, thereby helping newborn animals to acquire passive immunity. FcRn (also designated FCGRT, Brambell receptor, FcRn α chain, IgG Gc receptor and neonatal Fc-receptor) is comprised of a heavy chain and β -2-Microglobulin. FcRn heavy chain shows approximately 35% amino acid identity to an MHC class I molecule. FcRn localizes in endosomes of vascular endothelial cells and selectively recycles IgG to the cell surface, thus protecting IgG from lysosomal catabolism. This protection mechanism is a major constituent for ensuring IgG are the longest lived of all plasma proteins.

REFERENCES

1. Claypool, S.M., et al. 2002. Functional reconstitution of human FcRn in Madin-Darby canine kidney cells requires co-expressed human β -2-Microglobulin. *J. Biol. Chem.* 277: 28038-28050.
2. Praetor, A., et al. 2002. Membrane-anchored human FcRn can oligomerize in the absence of IgG. *J. Mol. Biol.* 321: 277-284.
3. Detmer, S.A., et al. 2002. IgG transcytosis and recycling by FcRn expressed in MDCK cells reveals ligand-induced redistribution. *EMBO J.* 21: 5953.
4. Zhou, J., et al. 2003. Generation of mutated variants of the human form of the MHC class I-related receptor, FcRn, with increased affinity for mouse immunoglobulin G. *J. Mol. Biol.* 332: 901-913.
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CHROMOSOMAL LOCATION

Genetic locus: FCGRT (human) mapping to 19q13.33.

PRODUCT

FcRn 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 FcRn siRNA (h): sc-45632 and FcRn shRNA Plasmid (h): sc-45632-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

FcRn shRNA (h) Lentiviral Particles is recommended for the inhibition of FcRn 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

FcRn (H-4): sc-166413 is recommended as a control antibody for monitoring of FcRn 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 FcRn gene expression knockdown using RT-PCR Primer: FcRn (h)-PR: sc-45632-PR (20 μ l, 599 bp). 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.

PROTOCOLS

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