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CD8- α shRNA (m) Lentiviral Particles: sc-43677-V

BACKGROUND

The T cell receptor (TCR) is a heterodimer composed of either α and β or γ and δ chains. CD3 chains and the CD4 or CD8 (CD8- α and CD8- β) co-receptors are also required for efficient signal transduction through the TCR. The TCR is expressed on T helper and T cytotoxic cells that can be distinguished by their expression of CD4 and CD8 proteins; T helper cells express CD4 proteins and T cytotoxic cells display CD8 proteins. CD8s are cell surface glycoproteins that exist as two chain complex ($\alpha\alpha$ or $\alpha\beta$) receptors that bind class I MHC molecules presented by the antigen-presenting cell (APC). A primary function of CD8 proteins is to facilitate antigen recognition by the TCR and to strengthen the avidity of the TCR-antigen interactions. An additional role for CD8-expressing T cells may be to maintain low levels of HIV expression.

REFERENCES

1. Nakayama, K., et al. 1989. Structure and expression of the gene encoding CD8 alpha chain (Leu-2/T8). *Immunogenetics* 30: 393-397.
2. Allison, J.P., et al. 1991. The immunobiology of T cells with invariant gamma delta antigen regions. *Ann. Rev. Immunol.* 9: 679-705.
3. Zuniga-Pflucker, J.C., et al. 1991. CD4 and CD8 act as co-receptors during thymic selection of the T cell repertoire. *Sem. Immunol.* 3: 167-175.
4. Fleury, S.G., et al. 1991. CD4 and CD8 recognition of class II and class I molecules of the major histocompatibility complex. *Sem. Immunol.* 3: 177-185.
5. Janeway, C.A., et al. 1992. The T cell receptor as a multicomponent signalling machine: CD4/CD8 coreceptors and CD45 in T cell activation. *Annu. Rev. Immunol.* 10: 645-674.
6. Julius, M., et al. 1993. Distinct roles for CD4 and CD8 as co-receptors in antigen receptor signalling. *Immunol. Today* 14: 177-183.

CHROMOSOMAL LOCATION

Genetic locus: Cd8a (mouse) mapping to 6 C1.

PRODUCT

CD8- α shRNA (m) 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 CD8- α siRNA (m): sc-43677 and CD8- α shRNA Plasmid (m): sc-43677-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

CD8- α shRNA (m) Lentiviral Particles is recommended for the inhibition of CD8- α expression in mouse 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

CD8- α (H-160): sc-7188 is recommended as a control antibody for monitoring of CD8- α 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 CD8- α gene expression knockdown using RT-PCR Primer: CD8- α (m)-PR: sc-43677-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.