

CD45 siRNA (h): sc-29251

BACKGROUND

CD45 has been identified as a transmembrane glycoprotein, broadly expressed among hematopoietic cells. Multiple isoforms of CD45 are distributed throughout the immune system according to cell type. These isoforms arise because of alternative splicing of exons 4, 5 and 6. The corresponding protein domains are characterized by the binding of monoclonal antibodies specific for CD45RA (exon 4), CD45RB (exon 5), CD45RC (exon 6) and CD45RO (exons 4 to 6 spliced out). The variation in these isoforms is localized to the extracellular domain of CD45, while the intracellular domain is conserved. CD45 functions as a phosphotyrosine phosphatase, a vital component for efficient tyrosine phosphorylation induction by the TCR/CD3 complex. The tyrosine phosphatase activity of CD45 is contained within the conserved intracellular domain. Src and Syk family protein tyrosine kinases are utilized by the TCR/CD3 complex to initiate signaling cascades. Several members of these two families, including Lck, Fyn and ZAP-70, have been implicated as physiological substrates of CD45.

REFERENCES

1. Trowbridge, I.S. 1978. Interspecies spleen-myeloma hybrid producing monoclonal antibodies against mouse lymphocyte surface glycoprotein, T200. *J. Exp. Med.* 148: 313-323.
2. West, K.P., et al. 1986. The demonstration of B cell, T cell and myeloid antigens in paraffin sections. *J. Pathol.* 150: 89-101.
3. Streuli, M., et al. 1987. Differential usage of three exons generates at least five different mRNAs encoding human leukocyte common antigens. *J. Exp. Med.* 166: 1548-1566.

CHROMOSOMAL LOCATION

Genetic locus: PTPRC (human) mapping to 1q31.3.

PRODUCT

CD45 siRNA (h) is a pool of 4 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CD45 shRNA Plasmid (h): sc-29251-SH and CD45 shRNA (h) Lentiviral Particles: sc-29251-V as alternate gene silencing products.

For independent verification of CD45 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29251A, sc-29251B, sc-29251C and sc-29251D.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

CD45 siRNA (h) is recommended for the inhibition of CD45 expression in human 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 μ M in 66 μ l. 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

CD45 (35-Z6): sc-1178 is recommended as a control antibody for monitoring of CD45 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 CD45 gene expression knockdown using RT-PCR Primer: CD45 (h)-PR: sc-29251-PR (20 μ l, 312 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Charpentier, X., et al. 2009. Chemical genetics reveals bacterial and host cell functions critical for type IV effector translocation by *Legionella pneumophila*. *PLoS Pathog.* 5: e1000501.

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.