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SANTA CRUZ BIOTECHNOLOGY, INC.

RAG-1 siRNA (m): sc-42963

BACKGROUND

Immunoglobulin (Ig) and the T cell receptor are the receptors of B and T lymphocytes and are encoded in multiple germ line DNA segments, V, D and J, that are rearranged during lymphocyte development. This is the only known example of site specific recombination in vertebrate genes. Several genes are essential for V(D)J rearrangement. The recombination activator genes RAG-1 and RAG-2 were originally identified on the basis of their ability to activate rearrangement of an exogenous recombinational substrate in fibroblasts; moreover, both genes are required for this activity. It is yet to be resolved as to whether RAG-1 and RAG-2 encode components of the V(D)J recombinase itself or regulatory proteins that potentiate V(D)J recombination.

REFERENCES

1. Schatz, D.G., et al. 1989. The V(D)J recombination activating gene, RAG-1. Cell 59: 1035-1048.
2. Schatz, D.G., et al. 1992. V(D)J recombination: molecular biology and regulation. Annu. Rev. Immunol. 10: 359-383.
3. Shinkai, Y., et al. 1992. RAG2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. Cell 68: 855-867.
4. Mombaerts, P., et al. 1992. RAG-1-deficient mice have no mature B and T lymphocytes. Cell 68: 869-877.
5. Lin, W., et al. 1993. Regulation of V(D)J recombination activator protein RAG-2 by phosphorylation. Science 260: 953-959.
6. Wang, L.C., et al. 1993. RAG-1 and RAG-2 are not sufficient to direct all phases of immunoglobulin gene rearrangement in pre-B-cell lines. Mol. Cell. Biol. 13: 3890-3899.
7. Chen, J., et al. 1993. RAG-2-deficient blastocyst complementation: an assay of gene function in lymphocyte development. Proc. Natl. Acad. Sci. USA 90: 4528-4532.

CHROMOSOMAL LOCATION

Genetic locus: Rag1 (mouse) mapping to 2 E2.

PRODUCT

RAG-1 siRNA (m) is a pool of 3 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RAG-1 shRNA Plasmid (m): sc-42963-SH and RAG-1 shRNA (m) Lentiviral Particles: sc-42963-V as alternate gene silencing products.

For independent verification of RAG-1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-42963A, sc-42963B and sc-42963C.

PROTOCOLS

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

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

RAG-1 siRNA (m) is recommended for the inhibition of RAG-1 expression in mouse 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 µM in 66 µl. 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

RAG-1 (D-5): sc-377127 is recommended as a control antibody for monitoring of RAG-1 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 RAG-1 gene expression knockdown using RT-PCR Primer: RAG-1 (m)-PR: sc-42963-PR (20 µl, 525 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.