SANTA CRUZ BIOTECHNOLOGY, INC.

Pegasus siRNA (h): sc-90477

BACKGROUND

Ikaros family members, including Ikaros and Helios, are nuclear factors that colocalize with DNA replication machinery components in higher-order chromatin structures and respond to signaling events, such as T-cell activation. Helios and Ikaros bind to similar DNA sequences and they function as hemopoietic-specific transcription factors. Members of the Ikaros family contain zinc-finger domains that are involved in DNA-binding and in the formation of homodimers and heterodimers between Ikaros family members. Pegasus, also known as zinc finger protein Ikaros 5, is a 419 amino acid transcriptional repressor. Localized to the nucleus, Pegasus binds to the 5'GNNTGTNG-3' DNA core sequence. Pegasus contains five C_2H_2-type zinc finger domains. The C-terminal zinc finger domains participate in homodimerization and the N-terminal domains participate in heterodimerization with Ikaros family members and DNA binding. Pegasus is expressed in many tissues including kidney, heart, skeletal muscle, brain and liver.

REFERENCES

1. Perdomo, J., et al. 2000. Eos and Pegasus, two members of the Ikaros family of proteins with distinct DNA binding activities. J. Biol. Chem. 275: 38347-38354.
2. Nishii, K., et al. 2002. Non-DNA-binding Ikaros isoform gene expressed in adult B-precursor acute lymphoblastic leukemia. Leukemia 16: 1285-1292.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606238. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Yoshida, T., et al. 2006. Early hematopoietic lineage restrictions directed by Ikaros. Nat. Immunol. 7: 382-391.
5. Ng, S.Y., et al. 2007. Ikaros and chromatin regulation in early hematopoiesis. Curr. Opin. Immunol. 19: 116-122.
6. Ronni, T., et al. 2007. Human Ikaros function in activated T cells is regulated by coordinated expression of its largest isoforms. J. Biol. Chem. 282: 2538-2547.
7. Zhu, X., et al. 2007. Ikaros is regulated through multiple histone modifications and deoxyribonucleic acid methylation in the pituitary. Mol. Endocrinol. 21: 1205-1215.

CHROMOSOMAL LOCATION

Genetic locus: IKZF5 (human) mapping to 10q26.13.

PRODUCT

Pegasus siRNA (h) 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 Pegasus shRNA Plasmid (h): sc-90477-SH and Pegasus shRNA (h) Lentiviral Particles: sc-90477-V as alternate gene silencing products.

For independent verification of Pegasus (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-90477A, sc-90477B and sc-90477C.

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

Pegasus siRNA (h) is recommended for the inhibition of Pegasus 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 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

Pegasus (1B6): sc-517137 is recommended as a control antibody for monitoring of Pegasus 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 Pegasus gene expression knockdown using RT-PCR Primer: Pegasus (h)-PR: sc-90477-PR (20 µl). 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.

PROTOCOLS

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