SANTA CRUZ BIOTECHNOLOGY, INC.

DAP10 siRNA (h): sc-35171

BACKGROUND

DAP10, a transmembrane type 1 protein, is predominantly expressed in hematopoietic cells. On SDS-PAGE, DAP10 migrates slightly slower than expected due to glycosylation. DAP10 forms an activating receptor complex with its physiological partner, NKG2D. NKG2D is an activating receptor that initiates Natural Killer and T-cell mediated cytotoxicity against tumors expressing its ligands MICA and MICB. The DAP10-NKG2D complex, as well as MICA and MICB, are stress-inducible molecules expressed in epithelial tumors. Both DAP10 and NKG2D contain inhibition motifs in their cytoplasmic domains that recruit tyrosine-phosphatases, resulting in the inactivation of Natural Killer cells. The cytoplasmic region of DAP10 also contains a binding site for the SH2 domain of the p85 subunit of PI 3-kinase which suggests a role for DAP10 as a signal transducer leading to PI 3-kinase activation.

REFERENCES

1. Songyang, Z., Shoelson, S., Chaudhuri, M., Gish, G., Pawson, T., Haser, W., King, F., Roberst, T., Ratnofsky, S. and Lechleider, R. 1993. SH2 domains recognize specific phosphopeptide sequences. Cell 72: 767-778.
2. Groh, V., Bahram, S., Bauer, S., Herman, A., Beauchamp, M. and Spies, T. 1996. Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. Proc. Natl. Acad. Sci. USA 93: 12445-12450.
3. Lanier, L., Corliss, B., Wu, J. and Phillips, J. 1998. Association of DAP12 with activating CD94/NKG2C NK cell receptors. Immunity 8: 693-701.
4. Bauer, S., Groh, V., Wu, J., Steinle, A., Phillips, J., Lanier, L. and Spies, J. 1999. Activation of NK cells and T-cells by NKG2D, a receptor for stress-inducible MICA. Science 285: 727-729.
5. Wu, J., Song, Y., Bakker, A., Bauer, S., Spies, T., Lanier, L. and Phillips, J. 1999. An activating immunoreceptor complex formed by NKG2D and DAP10. Science 285: 730-732.

CHROMOSOMAL LOCATION

Genetic locus: HCST (human) mapping to 19q13.12.

PRODUCT

DAP10 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 DAP10 shRNA Plasmid (h): sc-35171-SH and DAP10 shRNA (h) Lentiviral Particles: sc-35171-V as alternate gene silencing products.

For independent verification of DAP10 (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-35171A, sc-35171B and sc-35171C.

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

DAP10 shRNA Plasmid (h) is recommended for the inhibition of DAP10 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

DAP10 (H-3): sc-374196 is recommended as a control antibody for monitoring of DAP10 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 DAP10 gene expression knockdown using RT-PCR Primer: DAP10 (h)-PR: sc-35171-PR (20 µl, 398 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.