SANTA CRUZ BIOTECHNOLOGY, INC.

Net siRNA (h): sc-37867

BACKGROUND

Ras signaling is mediated in part by transcription factors, which belong to one of the largest families of signal-dependent transcriptional regulators, the Ets gene family. One member of the Ets gene family, Net (also designated ERP and SAP-2), shares various properties with Ets factors Elk-1 and SAP-1. Like Ets factors Elk-1 and SAP-1, Net binds to Ets DNA motifs through the Ets domain and forms ternary complexes with the serum response factor SRF on the Fos serum response element SRE. Net contains two nuclear localization signals, one in the Ets domain and one corresponding to the D box, as well as a nuclear export signal in the conserved Ets DNA binding domain. Net is exported from the nucleus in response to stress stimuli transduced through the JNK pathway. ERK and p38 bind to the D box of Net to allow phosphorylation of the adjacent C-terminal C-domain, which, in combination with the D box, is required for transcription activation by Ras. However, the binding of JNK to the J box results in phosphorylation of the adjacent export motif, which is important for Net export from the nucleus. Therefore, Net acts as a transcriptional repressor that is converted into an activator by Ras/ERK signaling and is regulated by nuclear-cytoplasmic shuttling in response to specific signaling pathways.

REFERENCES

1. Giovane, A., et al. 1994. Net, a new Ets transcription factor that is activated by Ras. Genes Dev. 8: 1502-1513.
2. Price, M.A., et al. 1995. Comparative analysis of the ternary complex factors Elk-1, SAP-1a and SAP-2 (ERP/Net). EMBO J. 14: 2589-2601.
3. Ducret, C., et al. 1999. The Net repressor is regulated by nuclear export in response to anisomycin, UV, heat shock. Mol. Cell. Biol. 19: 7076-7087.
4. Ducret, C., et al. 2000. The ternary complex factor Net contains two distinct elements that mediate different responses to MAP kinase signaling cascades. Oncogene 19: 5063-5072.
5. Mavrothalassitis, G., et al. 2000. Proteins of the Ets family with transcriptional repressor activity. Oncogene 19: 6524-6532.
6. Zheng, H., et al. 2003. The transcription factor Net regulates the angiogenic switch. Genes Dev. 17: 2283-2297.

CHROMOSOMAL LOCATION

Genetic locus: ELK3 (human) mapping to 12q23.1.

PRODUCT

Net 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 Net shRNA Plasmid (h): sc-37867-SH and Net shRNA (h) Lentiviral Particles: sc-37867-V as alternate gene silencing products.

For independent verification of Net (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-37867A, sc-37867B and sc-37867C.

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

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

Net (11G9): sc-134401 is recommended as a control antibody for monitoring of Net 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 Net gene expression knockdown using RT-PCR Primer: Net (h)-PR: sc-37867-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.