



## Net siRNA (m): sc-37868

### 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.

### CHROMOSOMAL LOCATION

Genetic locus: ELK3 (human) mapping to 12q23; Elk3 (mouse) mapping to 10 C-D1.

### PRODUCT

Net siRNA (m) is a pool of 3 target-specific 20-25 nt siRNAs designed to knock down gene expression. Each vial contains 3 nmol of lyophilized siRNA, sufficient for a 10  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections.

For independent verification of Net (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-37868A, sc-37868B and sc-37868C.

### PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

Net siRNA (m) is recommended for the inhibition of Net expression in mouse cells.

Net (C-20): sc-17860 is recommended as a control antibody for Western Blotting (starting dilution 1:100, dilution range 1:100-1:1,000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) protein detection using the recommended secondary reagents listed below.

### RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

### 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  $\mu$ M in 60  $\mu$ 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. Semi-quantitative RT-PCR may be performed using RT-PCR Primer: Net (m)-PR: sc-37868-PR (20  $\mu$ l).

### RESEARCH USE

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