SANTA CRUZ BIOTECHNOLOGY, INC.

IEX-1 siRNA (m): sc-146148

BACKGROUND

Tumors are frequently observed as resistant to apoptotic induction by FAS, tumor necrosis factor α (TNF-α) or irradiation. This anti-death activity may be attributed to immediate early-response genes that are regulated at the transcriptional level, including the protein IEX-1. IEX-1 (immediately early gene X-1), also known as IER3 (immediate early response 3), DIF-2 (differentiation-dependent gene 2 protein), immediate early protein GLY96 or PRG1 (PACAP-responsive gene 1 protein), is a 156 amino acid single-pass type II membrane protein that belongs to the IER3 family. IEX-1 was originally characterized as a gene induced by ultraviolet radiation and TNF-α, which protected human squamous carcinoma cells from apoptosis. Subsequent transfection studies have also shown that expression of IEX-1 in human keratinocytes and mouse fibroblasts results in more rapid proliferation of the cells as compared with controls. The promoter region of IEX-1 contains binding motifs for both NFκB and p53, suggesting that these proteins may regulate its expression.

REFERENCES

1. Beyaert, R., et al. 1994. Molecular mechanisms of tumor necrosis factor-induced cytotoxicity. What we do understand and what we do not. FEBS Lett. 340: 9-16.
2. Kondratyev, A.D., et al. 1996. Identification and characterization of a radiation-inducible glycosylated human early-response gene. Cancer Res. 56: 1498-1502.
3. Wu, M.X., et al. 1998. IEX-1L, an apoptosis inhibitor involved in NFκB-mediated cell survival. Science 281: 998-1001.
4. Kumar, R., et al. 1998. A novel immediate early response gene, IEX-1, is induced by ultraviolet radiation in human keratinocytes. Biochem. Biophys. Res. Commun. 253: 336-341.
5. Schafer, H., et al. 1998. The promoter of human p22/PACAP response gene 1 (PRG1) contains functional binding sites for the p53 tumor suppressor and for NFκB. FEBS Lett. 436: 139-143.

CHROMOSOMAL LOCATION

Genetic locus: Ier3 (mouse) mapping to 17 B1.

PRODUCT

IEX-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 IEX-1 shRNA Plasmid (m): sc-146148-SH and IEX-1 shRNA (m) Lentiviral Particles: sc-146148-V as alternate gene silencing products.

For independent verification of IEX-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-146148A, sc-146148B and sc-146148C.

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

IEX-1 siRNA (m) is recommended for the inhibition of IEX-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

IEX-1 (E-2): sc-515605 is recommended as a control antibody for monitoring of IEX-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 IEX-1 gene expression knockdown using RT-PCR Primer: IEX-1 (m)-PR: sc-146148-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.