

FAST siRNA (h): sc-35361

BACKGROUND

Fas, also referred to as CD95 or APO-1, is a type I transmembrane protein that plays a central role mediating viral immunity. TIA-1 and TIAR are two closely related proteins that possess three RRM (RNA recognition motifs), designated RRM 1, 2 and 3, respectively. Although both TIA-1 and TIAR are thought to function as mediators of apoptotic cell death, their specific roles in such pathways are unknown. Unlike TIA-1, which is found in the granules of cytotoxic lymphocytes, TIAR expression is limited to the nucleus and found in a much broader range of cells including, but not limited to, cells of hematopoietic origin. TIAR is translocated to the cytoplasm shortly after Fas ligation and this event immediately proceeds the onset of DNA fragmentation. A novel serine/threonine kinase that is activated as a result of Fas ligation, designated FAST (Fas-activated serine/threonine), shows kinase specificity towards both TIA-1 and TIAR. In unstimulated Jurkat cells, FAST resides in the cytoplasm as a highly phosphorylated protein and is quickly dephosphorylated and activated in response to stimulated Fas.

REFERENCES

1. Hanabuchi, S., et al. 1994. Fas and its ligand in a general mechanism of T-cell-mediated cytotoxicity. *Proc. Natl. Acad. Sci. USA* 91: 4930-4934.
2. Taupin, J.L., et al. 1995. The RNA-binding protein TIAR is translocated from the nucleus to the cytoplasm during Fas-mediated apoptotic cell death. *Proc. Natl. Acad. Sci. USA* 92: 1629-1633.
3. Visonneau, S., et al. 1995. A revertant TCR $\gamma\delta^+$ cell clone which has lost MHC nonrestricted cytotoxic activity but retains redirected killing upon stimulation of the CD3 receptor. *Cell. Immunol.* 165: 252-265.
4. Anderson, P. 1995. TIA-1: structural and functional studies on a new class of cytolytic effector molecule. *Curr. Top. Microbiol. Immunol.* 198: 131-143.
5. Tian, Q., et al. 1995. Fas-activated serine/threonine kinase (FAST) phosphorylates TIA-1 during Fas-mediated apoptosis. *J. Exp. Med.* 182: 865-874.
6. Dember, L.M., et al. 1996. Individual RNA recognition motifs of TIA-1 and TIAR have different RNA binding specificities. *J. Biol. Chem.* 271: 2783-2788.
7. Li, W., et al. 2004. FAST is a Bcl-X_L-associated mitochondrial protein. *Biochem. Biophys. Res. Commun.* 318: 95-102.

CHROMOSOMAL LOCATION

Genetic locus: FASTK (human) mapping to 7q36.1.

PRODUCT

FAST 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see FAST shRNA Plasmid (h): sc-35361-SH and FAST shRNA (h) Lentiviral Particles: sc-35361-V as alternate gene silencing products.

For independent verification of FAST (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-35361A, sc-35361B and sc-35361C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

FAST siRNA (h) is recommended for the inhibition of FAST 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 μ M in 66 μ 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.

GENE EXPRESSION MONITORING

FAST (B-9): sc-365125 is recommended as a control antibody for monitoring of FAST 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 FAST gene expression knockdown using RT-PCR Primer: FAST (h)-PR: sc-35361-PR (20 μ l). 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.