

TRAF6 siRNA (r): sc-156004

BACKGROUND

Tumor necrosis factor receptor-associated factor 6 (TRAF6) regulates adaptive immunity, innate immunity and bone metabolism. TRAF6 is a ubiquitin (Ub) ligase that mediates the activation of protein kinases, such as transforming growth factor β -activated kinase (TAK1) and $\text{I}\kappa\text{B}$ kinase (IKK), by catalyzing the formation of a unique polyubiquitin chain linked through Lys 63 of Ub. TRAF6 is essential for activating NF κ B signaling pathway in response to interleukin-1 and Toll-like receptor ligands. The coiled-coil domain of TRAF6 is essential for its auto-ubiquitination and activating NF κ B signaling pathway. TRAF6 interacts with various protein kinases including IRAK1/IRAK, SRC and PKC ζ , which provides a link between distinct signaling pathways.

CHROMOSOMAL LOCATION

Genetic locus: Traf6 (rat) mapping to 3q31.

PRODUCT

TRAF6 siRNA (r) 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 TRAF6 shRNA Plasmid (r): sc-156004-SH and TRAF6 shRNA (r) Lentiviral Particles: sc-156004-V as alternate gene silencing products.

For independent verification of TRAF6 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-156004A, sc-156004B and sc-156004C.

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

TRAF6 siRNA (r) is recommended for the inhibition of TRAF6 expression in rat 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

TRAF6 (D-10): sc-8409 is recommended as a control antibody for monitoring of TRAF6 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 MarkerTM 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 TRAF6 gene expression knockdown using RT-PCR Primer: TRAF6 (r)-PR: sc-156004-PR (20 μl , 416 bp). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

SELECT PRODUCT CITATIONS

- Cao, Q., et al. 2015. TNF receptor-associated factor 6 (TRAF6) mediates the angiotensin-induced non-canonical TGF- β pathway activation of c-Kit⁺ cardiac stem cells. *Am. J. Transl. Res.* 7: 2233-2243.
- Huang, W., et al. 2016. Combination of microRNA-21 and microRNA-146a attenuates cardiac dysfunction and apoptosis during acute myocardial infarction in mice. *Mol. Ther. Nucleic Acids* 5: e296.
- Yin, Y., et al. 2016. MiR-146a regulates inflammatory infiltration by macrophages in polymyositis/dermatomyositis by targeting TRAF6 and affecting IL-17/ICAM-1 pathway. *Cell. Physiol. Biochem.* 40: 486-498.
- Jia, L., et al. 2016. MicroRNA 146a locally mediates distal axonal growth of dorsal root ganglia neurons under high glucose and sildenafil conditions. *Neuroscience* 329: 43-53.
- Nishizaki, T. 2018. IL-33 suppresses GSK-3 β activation through an ST2-independent MyD88/TRAF6/RIP/PI3K/Akt pathway. *Heliyon* 4: e00971.
- Jiao, M., et al. 2019. Effect of the SSeCKS-TRAF6 interaction on gastrodin-mediated protection against 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced astrocyte activation and neuronal death. *Chemosphere* 226: 678-686.
- Cao, Y., et al. 2020. BAFF is involved in the pathogenesis of IgA nephropathy by activating the TRAF6/NF κ B signaling pathway in glomerular mesangial cells. *Mol. Med. Rep.* 21: 795-805.

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.