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TRAF6 shRNA (h2) Lentiviral Particles: sc-44329-V

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 I κ 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.

REFERENCES

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- Ea, C.K., et al. 2004. TIFA activates I κ B kinase (IKK) by promoting oligomerization and ubiquitination of TRAF6. *Proc. Nat. Acad. Sci. USA* 101: 15318-15323.
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- Manna, S.K., et al. 2005. Interleukin-8 induces nuclear transcription factor- κ B through a TRAF6-dependent pathway. *J. Biol. Chem.* 280: 7010-7021.
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CHROMOSOMAL LOCATION

Genetic locus: TRAF6 (human) mapping to 11p12.

PRODUCT

TRAF6 shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see TRAF6 siRNA (h2): sc-44329 and TRAF6 shRNA Plasmid (h2): sc-44329-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

TRAF6 shRNA (h2) Lentiviral Particles is recommended for the inhibition of TRAF6 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TRAF6 gene expression knockdown using RT-PCR Primer: TRAF6 (h2)-PR: sc-44329-PR (20 μ l, 598 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.