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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

RANK siRNA (h): sc-42960

BACKGROUND

Members of the tumor necrosis factor (TNF) receptor superfamily interact with signaling molecules of the TNF receptor-associated factor (TRAF) family to activate the NF κ B and JNK pathways. RANK (receptor activator of NF κ B) is a member of the TNFR family identified on dendritic cells. This type I membrane receptor is expressed in a broad range of tissues. The C-terminus of RANK is required for RANK to bind TRAF2, 5 and 6, and it is also necessary for stimulating NF κ B activation. The ligand for this receptor, RANKL (also designated TRANCE or ODF), is a type II transmembrane protein expressed primarily in lymphoid tissues and T cell lines. RANKL appears to be an important regulator of T cells and osteoclasts.

REFERENCES

1. Wong, B.R., et al. 1997. TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. *J. Biol. Chem.* 272: 25190-25194.
2. Natoli, G., et al. 1997. Tumor necrosis factor (TNF) receptor 1 signaling downstream of TNF receptor-associated factor 2. Nuclear factor κ B (NF κ B)-inducing kinase requirement for activation of activating protein 1 and NF κ B but not of c-Jun N-terminal kinase/stress-activated protein kinase. *J. Biol. Chem.* 272: 26079-26082.
3. Shi, C.S., et al. 1997. Activation of stress-activated protein kinase/c-Jun N-terminal kinase, but not NF κ B, by the tumor necrosis factor (TNF) receptor 1 through a TNF receptor-associated factor 2- and germinal center kinase related-dependent pathway. *J. Biol. Chem.* 272: 32102-32107.
4. Anderson, D.M., et al. 1997. A homologue of the TNF receptor and its ligand enhance T cell growth and dendritic cell function. *Nature* 390: 175-179.
5. Darnay, B.G., et al. 1998. Characterization of the intracellular domain of receptor activator of NF κ B (RANK). Interaction with tumor necrosis factor receptor-associated factors and activation of NF κ B and c-Jun N-terminal kinase. *J. Biol. Chem.* 273: 20551-20555.
6. Wong, B.R., et al. 1998. The TRAF family of signal transducers mediates NF κ B activation by the TRANCE receptor. *J. Biol. Chem.* 273: 28355-28359.

CHROMOSOMAL LOCATION

Genetic locus: TNFRSF11A (human) mapping to 18q21.33.

PRODUCT

RANK 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 RANK shRNA Plasmid (h): sc-42960-SH and RANK shRNA (h) Lentiviral Particles: sc-42960-V as alternate gene silencing products.

For independent verification of RANK (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-42960A, sc-42960B and sc-42960C.

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

RANK siRNA (h) is recommended for the inhibition of RANK 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

RANK (H-7): sc-374360 is recommended as a control antibody for monitoring of RANK 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 RANK gene expression knockdown using RT-PCR Primer: RANK (h)-PR: sc-42960-PR (20 μ l, 452 bp). 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.