Product Datasheet

RANK/TNFRSF11A Antibody (9A725) - BSA Free
NB100-56508

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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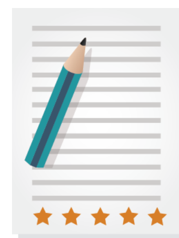


NB100-56508

RANK/TNFRSF11A Antibody (9A725) - BSA Free

Product Information

Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	9A725
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS

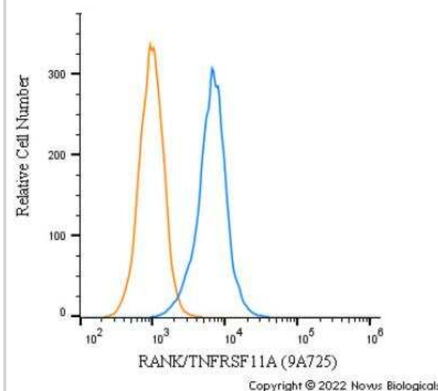
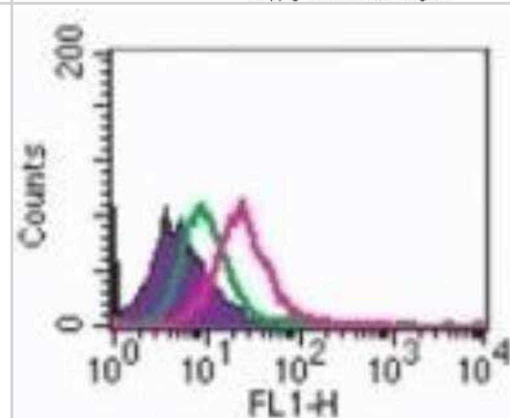
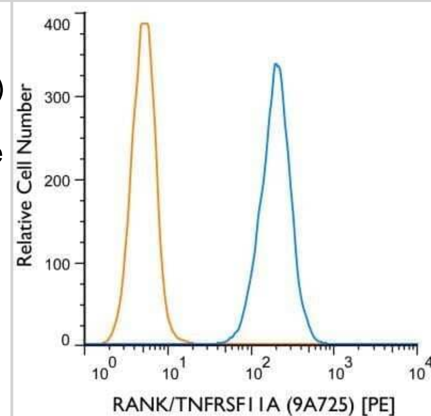
Product Description

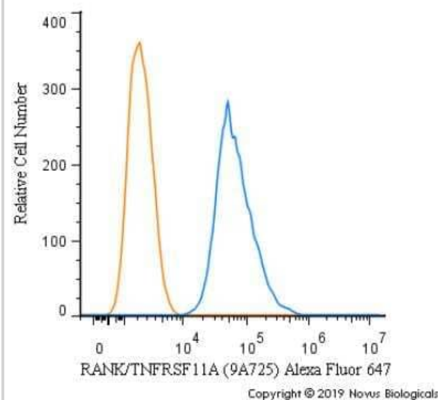
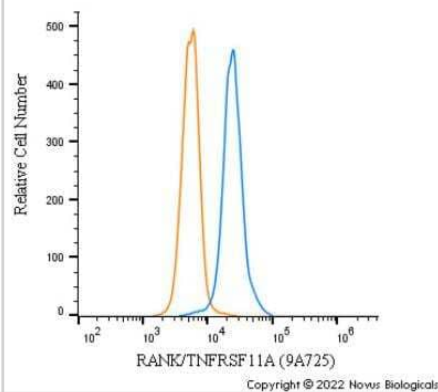
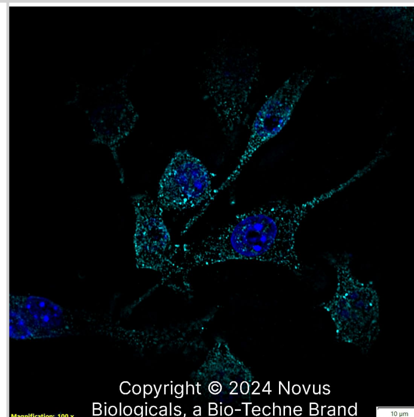
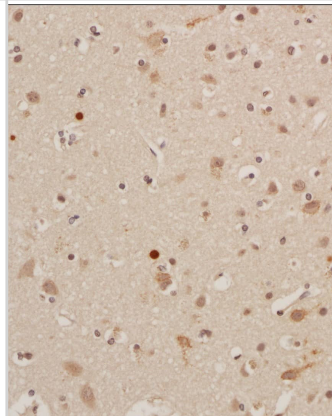
Description	Novus Biologicals Mouse RANK/TNFRSF11A Antibody (9A725) - BSA Free (NB100-56508) is a monoclonal antibody validated for use in IHC, WB and Flow. Anti-RANK/TNFRSF11A Antibody: Cited in 19 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	8792
Gene Symbol	TNFRSF11A
Species	Human, Mouse, Rat, Avian
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 24376119). Avian reactivity reported in scientific literature (PMID: 31214852).
Immunogen	A fusion protein containing amino acid residues 326-616 of human RANK was used as immunogen. This antibody recognizes an epitope located between amino acid residues 330-427 of human RANK (NP_003830).

Product Application Details

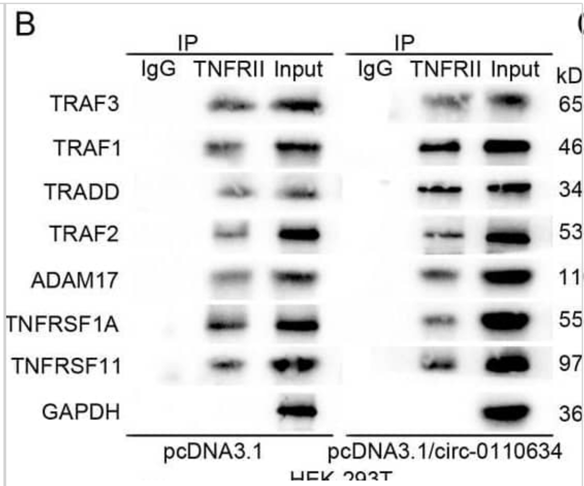
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Flow (Intracellular), Immunohistochemistry, CyTOF-ready
Recommended Dilutions	Western Blot 1-2 ug/ml, Flow Cytometry 2-4 ug/ml, Immunohistochemistry 1:10-1:500, Immunohistochemistry-Paraffin 1:10-1:500, Flow (Intracellular) 2 - 4 ug/ml, CyTOF-ready
Application Notes	Bharti et al (2004) published the RANK antibody clone 9A725 for Flow (Cell Surface). However, the epitope range recognized by this antibody is a potential cytoplasmic region as defined by www.uniprot.org. Hence, researchers are encouraged to refer to both the publication and the bioinformatics data bases for additional information in order to make their own determination regarding the suitability of the antibody for cell surface flow cytometry. In RAW cells, a 97 kDa is observed. This antibody is CyTOF ready.

Images

Western Blot: RANK/TNFRSF11A Antibody (9A725) [NB100-56508] - Analysis of RANK in transfected 293 cells using RANK antibody at 2 ug/ml.	
Flow Cytometry: RANK/TNFRSF11A Antibody (9A725) - BSA Free [NB100-56508] - An intracellular stain was performed on Raw264.7 cells with RANK/TNFRSF11A Antibody (9A725) NB100-56508 (blue) and a matched isotype control MAB002 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (84540, Thermo Fisher).	
Flow Cytometry: RANK/TNFRSF11A Antibody (9A725) [NB100-56508] - Intracellular flow cytometry analysis of RANK in 10^6 RAW cells using 2 ug of RANK antibody. Shaded histogram represents RAW cells without antibody; green represents isotype control; antibody red represents RANK antibody.	
Flow Cytometry: RANK/TNFRSF11A Antibody (9A725) [NB100-56508] - Using the PE direct conjugate An intracellular stain was performed on K-562 cells with RANK/TNFRSF11A (9A725) antibody NB100-56029 (blue) and a matched isotype control NBP1-97005PE (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.	

Flow Cytometry: RANK/TNFRSF11A Antibody (9A725) [NB100-56508] - An intracellular stain was performed on Raw264.7 cells with RANK/TNFRSF11A [9A725] Antibody NB100-56508AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	
Flow Cytometry: RANK/TNFRSF11A Antibody (9A725) - BSA Free [NB100-56508] - An intracellular stain was performed on MG-63 cells with RANK/TNFRSF11A Antibody (9A725) NB100-56508 (blue) and a matched isotype control MAB002 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (84540, Thermo Fisher).	
RANK/TNFRSF11A (9A725) was detected in immersion fixed Raw 264.7 mouse macrophage cell line using Mouse anti-RANK/TNFRSF11A (9A725) Protein-G purified Monoclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NB100-56508AF647) (light blue) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.	
Analysis of a FFPE tissue section of human brain using 1:200 dilution of RANK/TNFRSF11A Antibody (9A725) antibody. The staining was developed using HRP labeled anti-mouse secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.	

Circ-0110634 bound to TNFRII and TRAF2 (A) Potential proteins which could interact with TNFRII (B) The interaction between these proteins and TNFRII in HEK-293T cells with pcDNA3.1/circ-0110634 (C) RNA pull down assay was taken to verify whether circ-0110634 could combine with TRAF2 (D–E) RNA-protein pull-down and RIP assays were taken to verify whether circ-0110634 could bind to TRAF2 and TNFRII (F) TNFRII protein was cut down into 6 pieces (G–H) RNA-protein pull-down assays and gel electrophoresis were taken to verify the specific part circ-0110634 could bind to TNFRII (I) TRAF2 protein was cut into 8 pieces (J–K) RNA-protein pull down assays and gel electrophoresis were performed to confirm the binding capacity between specific pieces of TRAF2 and circ-0110634 (L) The binding capacity between TRAF2 and TNFRII was analyzed in circ-0110634-upregulated cells. □□P < 0.01 Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/36185580), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Li H, Liu C, Zheng S et al. RANKL/PD-1 dual blockade demonstrates survival benefit for patients with advanced lung adenocarcinoma harboring KRAS mutations. Cell Reports Medicine 2025-07-16 [PMID: 40669444]

W Ji, Y Lu, Z Ma, K Gan, Y Liu, Y Cheng, J Xu, S Liu, Y Guo, S Han, Z Zhao, H Xu, W Qi Triptolide attenuates inhibition of ankylosing spondylitis-derived mesenchymal stem cells on the osteoclastogenesis through modulating exosomal transfer of circ-0110634 Journal of Orthopaedic Translation, 2022-09-16;36(0):132-144. 2022-09-16 [PMID: 36185580]

Dorchin-Ashkenazi H, Ginat-Koton R, Gabet Y et al. The Balance between Orthodontic Force and Radiation in the Jawbone: Microstructural, Histological, and Molecular Study in a Rat Model Biology (Basel) 2021-11-18 [PMID: 34827196] (IHC-P, Rat)

Details:
Citation using the HRP format of this antibody.

Ferguson J, Wilcock DJ, McEntegart S et al. Osteoblasts contribute to a protective niche that supports melanoma cell proliferation and survival Pigment Cell Melanoma Res 2019-07-19 [PMID: 31323160] (WB, Human)

Hiyama S, Yokoi M, Akagi Y et al. Osteoclastogenesis from bone marrow cells during estrogen-induced medullary bone formation in Japanese quails J. Mol. Histol. 2019-08-01 [PMID: 31214852] (Avian)

Details:
Quail

Nanke Y, Kobashigawa T, Yago T et al. RANK Expression and Osteoclastogenesis in Human Monocytes in Peripheral Blood from Rheumatoid Arthritis Patients. BioMed Research International 2016-09-01 [PMID: 27822475] (FLOW, Human)

Huynh N, VonMoss L, Smith D et al. Characterization of Regulatory Extracellular Vesicles from Osteoclasts. J Dent Res 2016-06-01 [PMID: 26908631]

Lafferty MK, Fantry L, Bryant J et al. Elevated suppressor of cytokine signaling-1 (SOCS-1): a mechanism for dysregulated osteoclastogenesis in HIV transgenic rats Pathog Dis 2013-12-09 [PMID: 24376119] (Flow Cyometry, Rat)

Watari K, Shibata T, Nabeshima H et al. Impaired differentiation of macrophage lineage cells attenuates bone remodeling and inflammatory angiogenesis in Ndrg1 deficient mice. Sci Rep. 2016-01-18 [PMID: 26778110] (WB, Mouse)

Details:
RANK antibody used for WB on BM-derived macrophages (BMDMs) from WT and KO mice at a dilution of 1:1000 (Figure 3h).

Kaneko YS, Ota A, Nakashima A et al. Lipopolysaccharide treatment arrests the cell cycle of BV-2 microglial cells in G1 phase and protects them from UV light-induced apoptosis. J Neural Transm. 2014-06-12 [PMID: 24919883] (WB, Mouse) Details: Fig 3: BV-2 immortalized mouse microglial cell line. Procaspase and active/cleaved caspase were observed at 32 kDa and 17 kDa respectively.
Ayon Haro ER, Ukai T, Yokoyama M et al. Locally administered interferon-gamma accelerates lipopolysaccharide-induced osteoclastogenesis independent of immunohistological RANKL upregulation. J Periodontal Res. 2011-06-01 [PMID: 21361961] Details: RANK-PE, clone 9A725 (IMG-128D). Flow (cell surface): Primary mouse bone marrow macrophages. By flow cytometry analysis, ~95% of the bone marrow macrophage population was positive for RANK and CD11b (data described on page 363). Note: The RANK-PE mAb was used to help characterize the primary bone macrophages isolated from mouse tibiae and femurs (see Material and Methods, page 363).
Das S, Sepahi I, Duthie A et al. RANk receptor oligomerisation in the regulation of NFkB signalling. J. Mol. Endocrinol. 2014-05-23 [PMID: 24859969] (WB, Mouse) Details: HeLa cells transfected with mouse WT-RANkmyc, W434X-RANkmyc or G280X-RANkmyc constructs, Fig 3Bi. The specifically of RANk mAb was validated by WB with recombinant expressed mouse WT-RANkmyc (80 kDa) and W434X-RANkmyc (55 kDa). The RANk mAb did not detect
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Procedures

Flow (Intracellular) Protocol for RANK/TNFRSF11A Antibody (NB100-56508)
Protocol for Flow Cytometry Intracellular Staining
Sample Preparation.
1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer.
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.
Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.
Protocol for Cytoplasmic Targets:
1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabilization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 10 minutes.
a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 10-minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amounts of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuge at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuge at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

Western Blot Protocol for RANK/TNFRSF11A Antibody (NB100-56508)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

Immunohistochemistry-Paraffin Protocol for RANK/TNFRSF11A Antibody (NB100-56508)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

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Products Related to NB100-56508

NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

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This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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