

# Product Datasheet

## STAT3 Antibody (9D8) NBP2-22471

Unit Size: 100 uL

Store at -20C. Avoid freeze-thaw cycles.

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**NBP2-22471****STAT3 Antibody (9D8)**

Product Information	
Unit Size	100 uL
Concentration	1 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	9D8
Preservative	0.05% Sodium Azide
Isotype	IgG2a
Purity	Protein A purified
Buffer	PBS with 1 mg/ml BSA

Product Description	
Host	Mouse
Gene ID	6774
Gene Symbol	STAT3
Species	Human, Mouse, Rat, Primate, Monkey
Reactivity Notes	Please note that this antibody is reactive to Mouse and derived from the same host, Mouse. Additional Mouse on Mouse blocking steps may be required for IHC and ICC experiments. Please contact Technical Support for more information.
Immunogen	Residues 655-770 from human recombinant protein expressed in bacteria

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Knockdown Validated
Recommended Dilutions	Western Blot 1:5000, Immunohistochemistry 1:1600, Immunocytochemistry/ Immunofluorescence 1:100, Immunoprecipitation 2 ug, Immunohistochemistry-Paraffin 1:1600, Chromatin Immunoprecipitation (ChIP) 1-3 ul, Knockdown Validated
Application Notes	WB from verified customer review.

**Images**

Western Blot: STAT3 Antibody (9D8) [NBP2-22471] - Human breast cancer cell MDA-MB-231 was treated with carboplatin for 72 hours and the expression of p-Stat3 at Y705 and total Stat3 were detected by western blot. From verified customer review.

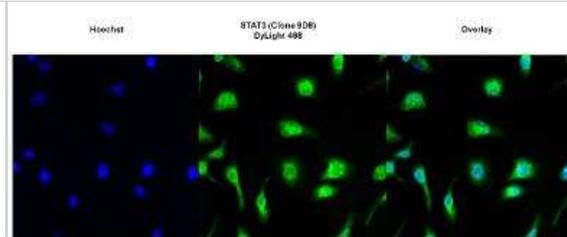
p-Stat3 at Y705



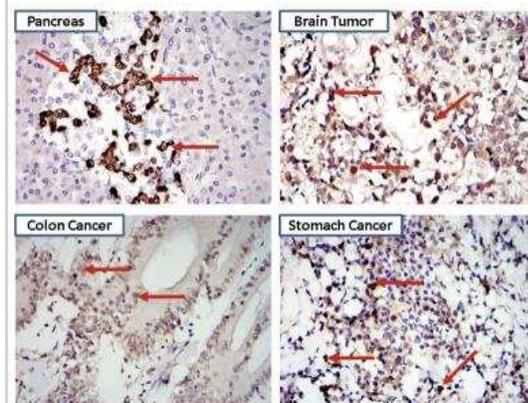
Stat3



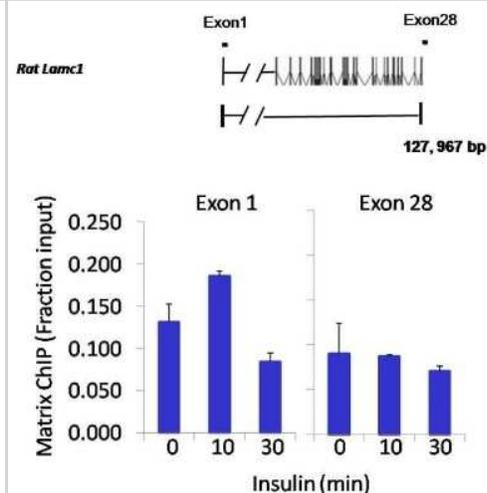
**Immunocytochemistry/Immunofluorescence: STAT3 Antibody (9D8) [NBP2-22471]** - Analysis of STAT3 using anti-STAT3 (9D8) monoclonal antibody (shown in green) in HeLa cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature. Cells were then blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with a mouse monoclonal antibody recognizing STAT3, at a dilution of 1:100 for at least 1 hour at room temperature. Cells were then washed with PBS and incubated with DyLight 488 goat-anti-mouse secondary antibody at a dilution of 1:400 for 30 minutes at room temperature. Nuclei (blue) were stained with Hoechst 33342 dye.



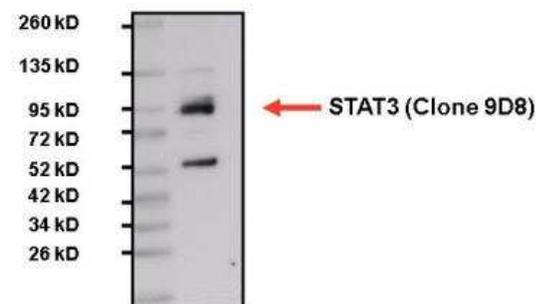
**Immunohistochemistry-Paraffin: STAT3 Antibody (9D8) [NBP2-22471]** - Biopsies of normal and cancer tissues.



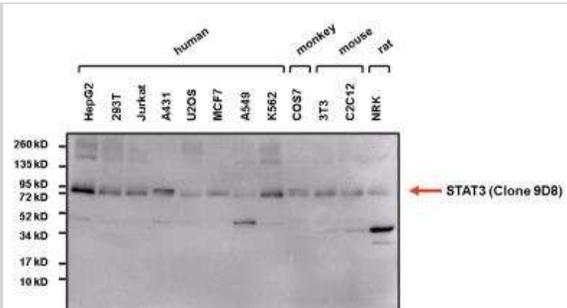
**Chromatin Immunoprecipitation: STAT3 Antibody (9D8) [NBP2-22471]** - Analysis performed using cross-linked chromatin from rat hepatoma cells treated with insulin. IP performed using a multiplex microplate Matrix ChIP assay with STAT3 monoclonal antibody. Chromatin aliquots from cells were used per ChIP pull-down. Quantitative PCR data done in quadruplicate using 1ul of DNA in 2ul SYBR real-time PCR reactions containing primers to amplify exon-1 or exon-28 of LAMC1. Quantitation of immunoprecipitated chromatin is presented as signal relative to the total amount of input chromatin. Results represent the mean +/- SEM for three experiments. A schematic representation of the rat LAMC1 locus is shown; boxes represent exons (black boxes = translated regions, white boxes = untranslated regions), the zigzag line represents an intron, and the straight line represents upstream sequence. Regions amplified by LAMC1 primers are represented by black bars. Data courtesy of the Innovators Program.



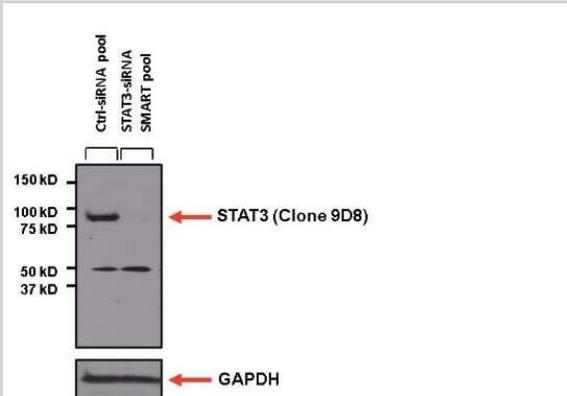
**Western Blot: STAT3 Antibody (9D8) [NBP2-22471]** - Analysis of 25ug HepG2 total lysate.



Western Blot: STAT3 Antibody (9D8) [NBP2-22471] - Analysis of 25ug of various whole cell lysates.



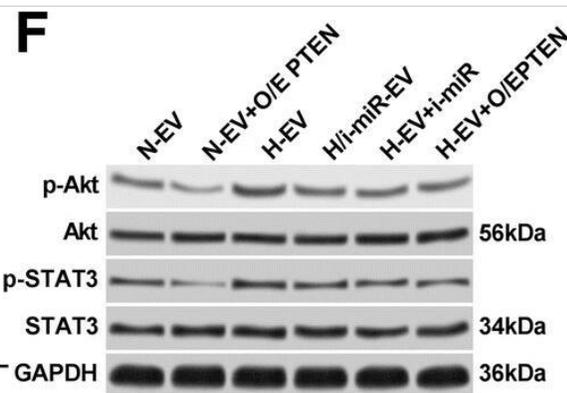
Western Blot: STAT3 Antibody (9D8) [NBP2-22471] - Analysis of 25ug of U2-OS lysate from STAT3 SMART pool siRNA transfected or non-targeting control transfected U2-OS cells onto a 4-20% Tris-HCl polyacrylamide gel.



Immunoprecipitation: STAT3 Antibody (9D8) [NBP2-22471] - Analysis of STAT3 was performed on HepG2 cells. The antigen: antibody complex was formed by incubating 750ug whole cell lysate with 2ug of mouse monoclonal antibody recognizing STAT3 overnight on a rocking platform at 4C. The immune-complex was then captured on 50ul Protein A/G Plus Agarose. Captured immune-complexes were then washed extensively and proteins eluted with 5X Reducing Sample Loading Dye. Samples were then resolved on a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to PVDF membrane and blocked with 5% Milk/TBS-0.1%Tween for at least 1 hour. Membranes were then probed with a mouse monoclonal antibody recognizing STAT3 at a dilution of 1:5000 overnight rotating at 4C. Membranes were washed in TBST and probed with Clean-blot IP detection reagent at a dilution of 1:2000 for at least one hour. Membranes were washed and chemiluminescent detection was performed using Super Signal West Dura.



N-EV & H-EV treatment promote macrophage M2 polarization by delivering miR-21-5p that targets PTEN. a, western blot analysis of PTEN protein expression level in induced macrophages. H/i-miR-EV, monocytes were induced with the presence of EV secreted by miR-21-5p-inhibited, hypoxia pre-challenged MSCs; H-EV + i-miR, monocytes were transfected with miR-21-5p inhibitor-expressing vector before induction with the presence of H-EV. Macrophages induced without MSC-EV were used as negative control (NC). b, c, flow cytometry determining the percentage of CD163+CD206+ cells among total CD68+ cells after induction. N-EV + O/E PTEN or H-EV + O/E PTEN, monocytes were transfected with PTEN overexpressing vector before N-EV or H-EV treatment, respectively. d–f, western blot detecting Akt & STAT3 protein expression as well as their activating phosphorylation (p-Ser473 for Akt & p-tyr705 for STAT3) in macrophages after induction. g–i, ELISA evaluating IL-10, TGF- $\beta$  & VEGF- $\alpha$  in macrophage culture medium after induction. Macrophages induced with the presence of N-EV were used as negative control in b–i. Tukey's test was used for statistical analysis. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$  Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30736829>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Zuo Q, Yang Y, Lyu Y, Yang C, Chen C, Salman S, Huang T, Wicks E, Jackson W, Datan E, Qin W, Semenza G. Plexin-B3 expression stimulates MET signaling, breast cancer stem cell specification, and lung metastasis. *Cell Rep*. 2023-02-28;42(3):112164. 2023-02-28 [PMID: 36857181]

Wareham LK, Echevarria FD, Sousa JL et al. Interleukin-6 promotes microtubule stability in axons via Stat3 protein-protein interactions *iScience* 2021-10-22 [PMID: 34646984]

Olszewska B, Zawrocki A, Lakomy J et al. Mapping signal transducer and activator of transcription (STAT) activity in different stages of mycosis fungoides and Sezary syndrome *Int. J. Dermatol.* 2020-07-08 [PMID: 32643174]

Slawinska M, Lakomy J, Biernat W et al. STAT3, STAT5A, STAT5B, STAT6 proteins are overexpressed in human basal cell carcinoma *Clin. Exp. Dermatol.* 2019-07-19 [PMID: 31323143] (IF/IHC, Human)

Ren W, Hou J, Yang C et al. Extracellular vesicles secreted by hypoxia pre-challenged mesenchymal stem cells promote non-small cell lung cancer cell growth and mobility as well as macrophage M2 polarization via miR-21-5p delivery *J. Exp. Clin. Cancer Res.* 2019-02-08 [PMID: 30736829] (WB, Human)



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### **Products Related to NBP2-22471**

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HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-96778	Mouse IgG2a Isotype Control (M2A)
H00006774-P01-10ug	Recombinant Human STAT3 GST (N-Term) Protein

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### **Limitations**

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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