# **Product Datasheet**

# TRIM8 Antibody - BSA Free NBP1-89776

Unit Size: 0.1 ml

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

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**Publications: 1** 

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#### NBP1-89776

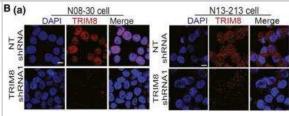
TRIM8 Antibody - BSA Free

I RIM8 Antibody - BSA Free	
Product Information	
Unit Size	0.1 ml
Concentration	Concentrations vary lot to lot. See vial label for concentration. If unlisted please contact technical services.
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS (pH 7.2) and 40% Glycerol
Product Description	
Host	Rabbit
Gene ID	81603
Gene Symbol	TRIM8
Species	Human
Immunogen	This antibody was developed against Recombinant Protein corresponding to amino acids: QDIEDQLYKLESDKRLVEEKVNQLKEEVRLQYEKLHQLLDEDLRQTVEVLDKA QAKFCSENAAQALHLGERMQEAKKLLGS
Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockdown Validated
Recommended Dilutions	Western Blot, Immunohistochemistry 1:50 - 1:200, Immunocytochemistry/ Immunofluorescence 0.25-2 ug/ml, Immunohistochemistry-Paraffin 1:50 - 1:200, Knockdown Validated
Application Notes	For IHC-Paraffin, HIER pH 6 retrieval is recommended. ICC/IF Fixation

Permeabilization: Use PFA/Triton X-100.

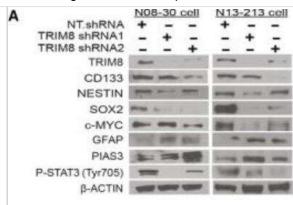
## **Images**

Immunocytochemistry/ Immunofluorescence: TRIM8 Antibody [NBP1-89776] - Knockdown of TRIM8 (GERP) promotes differentiation and impairs GBM stemness and self-renewal capacity. ICC shows reduced expression of TRIM8 (red), NESTIN (white), and SOX2 (red) in TRIM8 shRNA1-knockdown cells compared to NT shRNA cells. Nuclei were counterstained with DAPI (blue). Scale bar = 10 um. Image collected and cropped by CiteAb from the following publication (https://doi.wiley.com/10.1002/1878-0261.12034) licensed under a CC-BY license.

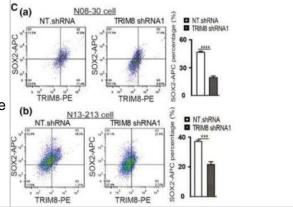




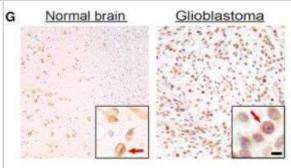
Western Blot: TRIM8 Antibody [NBP1-89776] - Knockdown of TRIM8 (GERP) promotes differentiation and impairs GBM stemness and self-renewal capacity. Western blot analysis shows reduced CD133, NESTIN, SOX2, and c-MYC, and upregulated GFAP in GBM neurosphere cells following TRIM8 knockdown by TRIM8 shRNA1 or TRIM8 shRNA2. NT shRNA = nontargeting control shRNA. Western blot also shows upregulated PIAS3 and reduced phosphorylated STAT3 (Tyr705) following shRNA knockdown of TRIM8. Image collected and cropped by CiteAb from the following publication (https://doi.wiley.com/10.1002/1878-0261.12034) licensed under a CC-BY license.



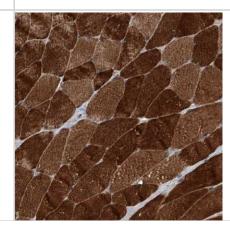
Flow Cytometry: TRIM8 Antibody [NBP1-89776] - Knockdown of TRIM8 promotes differentiation and impairs GBM stemness and self-renewal capacity. Flow cytometric analysis and statistical analysis of SOX2 expression in TRIM8-overexpressing neurosphere cells. SOX2 was tagged by APC (blue: 676 nm) with TRIM8 tagged by PE (red: 555 nm). \*\*\*P < 0.001, \*\*\*\*P < 0.0001. Statistics: Data are means +/- SD (n = 3), by nonparametric t-test. Image collected and cropped by CiteAb from the following publication (https://doi.wiley.com/10.1002/1878-0261.12034) licensed under a CC-BY license.



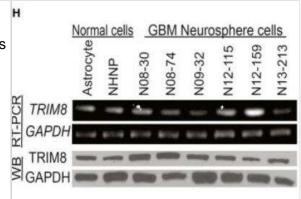
Immunohistochemistry: TRIM8 Antibody [NBP1-89776] - TRIM8 is expressed in GBM samples and neurosphere cells. Immunohistochemical (IHC) staining of TRIM8 in normal brain and GBM tissues. TRIM8 (+) cells (indicated by arrow) are predominantly neurons in the normal brain and show cytoplasmic staining. TRIM8 expression in GBM is predominant in nuclei of tumor cells with modest cytoplasmic staining. Sections were counterstained with hematoxylin. Scale bar = 10 um. Image collected and cropped by CiteAb from the following publication (https://doi.wiley.com/10.1002/1878-0261.12034) licensed under a CC-BY license.



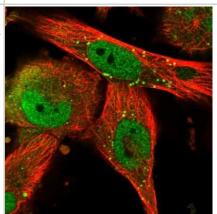
Immunohistochemistry-Paraffin: TRIM8 Antibody [NBP1-89776] - Staining of human skeletal muscle shows cytoplasmic positivity in myocytes.



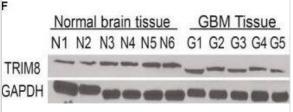
Western Blot: TRIM8 Antibody [NBP1-89776] - TRIM8 is expressed in GBM samples and neurosphere cells. Reverse transcription PCR analysis and western blot analysis of TRIM8 among two normal cell lines and six patient-derived GBM neurosphere cells. NHNP = normal human neural progenitor cell. Image collected and cropped by CiteAb from the following publication (https://doi.wiley.com/10.1002/1878-0261.12034) licensed under a CC-BY license.



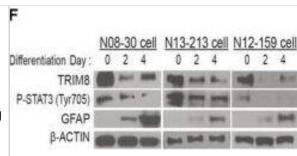
Immunocytochemistry/Immunofluorescence: TRIM8 Antibody [NBP1-89776] - Immunofluorescent staining of human cell line U-251 MG shows localization to nucleoplasm & vesicles.



Western Blot: TRIM8 Antibody [NBP1-89776] - TRIM8 is expressed in GBM samples & neurosphere cells. (A-C) Gene expression correlation of TRIM8 with STAT3, SOX2, & NESTIN using U133 mRNA data from TCGA. TRIM8 vs STAT3: Pearson's correlation coefficient: r = 0.36, P < 0.00001. TRIM8 vs SOX2: Pearson's correlation coefficient: r = 0.22, P < 0.00001. TRIM8 vs NESTIN: Pearson's correlation coefficient: r = 0.22, P < 0.00001. (D) Schematic representation of TRIM8 protein with RING, B box, & coiled coil domains. (E) TRIM8 copy number variation among 577 GBM samples in TCGA dataset. TRIM8 shows hemizvgous deletion in 88.04% of cases. (F) Western blot analysis of TRIM8 protein among six normal human brain tissues (N1–N6) & five GBMs (G1–G5). (G) Immunohistochemical (IHC) staining of TRIM8 in normal brain & GBM tissues. TRIM8 (+) cells (indicated by arrow) are predominantly neurons in the normal brain & show cytoplasmic staining. TRIM8 expression in GBM is predominant in nuclei of tumor cells with modest cytoplasmic staining. Sections were counterstained with hematoxylin. Scale bar = 10 µm. (H) Reverse transcription PCR analysis & western blot analysis of TRIM8 among two normal cell lines & six patient derived GBM neurosphere cells. NHNP = normal human neural progenitor cell. (I) Immunocytochemical (ICC) staining of TRIM8 in neurosphere cells showing nuclear expression of TRIM8. TRIM8 was labeled in GFP, & nuclei were counterstained with DAPI (blue). Scale bar = 10 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28100038), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: TRIM8 Antibody [NBP1-89776] - Knockdown of TRIM8 promotes differentiation & impairs GBM stemness & self renewal capacity. (A) Western blot analysis shows reduced CD133, NESTIN, SOX2, & c□MYC, & upregulated GFAP in GBM neurosphere cells following TRIM8 knockdown by TRIM8 shRNA1 or TRIM8 shRNA2. NT shRNA = nontargeting control shRNA. Western blot also shows upregulated PIAS3 & reduced phosphorylated STAT3 (Tyr705) following shRNA knockdown of TRIM8. (B) ICC shows reduced expression of TRIM8 (red), NESTIN (white), & SOX2 (red) in TRIM8 shRNA1 knockdown cells compared to NT shRNA cells. Nuclei were counterstained with DAPI (blue). Scale bar = 10 µm. (C) Flow cytometric analysis & statistical analysis of SOX2 expression in TRIM8 overexpressing neurosphere cells. SOX2 was tagged by APC (blue: 676 nm) with TRIM8 tagged by PE (red: 555 nm). \*\*\*P < 0.001. \*\*\*\*P < 0.0001. Statistics: Data are means  $\pm$  SD (n = 3), by nonparametric t test. (D,E) TRIM8 knockdown impairs neurosphere formation. Representative images of neurosphere expressing NT shRNA & TRIM8 shRNA1 at specific time points are shown. Scale bar = 30  $\mu$ m. Statistical analysis of average sphere size: by one way analysis of variance (ANOVA). Data are means  $\pm$  standard deviation (SD) (n = 6). \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001. (F) Western blot analysis shows reduced TRIM8 & activated STAT3 (Tyr705), with increased GFAP expression following differentiation induced by serum. (G) ICC reveals reduced TRIM8 (red) & increased GFAP (white) after four days of differentiation induced by serum. Nuclei were counterstained with DAPI (blue). Scale bar = 10 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28100038). licensed under a CC-BY license. Not internally tested by Novus Biologicals.

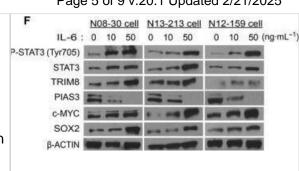


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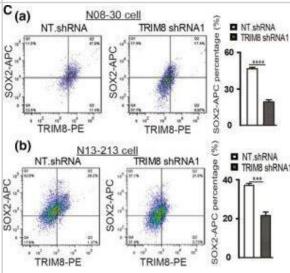




Western Blot: TRIM8 Antibody [NBP1-89776] - TRIM8 modulates GBM stemness via a positive loop of PIAS3 STAT3 pathway. (A) Western blot analysis of PIAS3 accumulation following TRIM8 overexpression in the presence of MG132 (20 µm) for four hours. (B-E) Western blot analyses showing that the half life of PIAS3 was reduced by TRIM8 overexpression & increased by TRIM8 knockdown after treating cells with 100 µg mL-1 cycloheximide (CHX) for 0, 2, 4, 6, & 8 h in two different neurosphere cell lines. (F) Western blot showing that interleukin 6 (IL□6; 10 & 50 ng·mL-1) induced phosphorylated STAT3 (Tyr705) & also enhanced expression of STAT3, c□MYC, SOX2, & TRIM8. (G) Western blot showing that the STAT3 Inhibitor XI (STX□0119; 10 & 100 µm) selectively suppresses phosphorylated STAT3 (Tyr705) & reduces expression of STAT3, c□MYC, SOX2, & TRIM8. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28100038), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

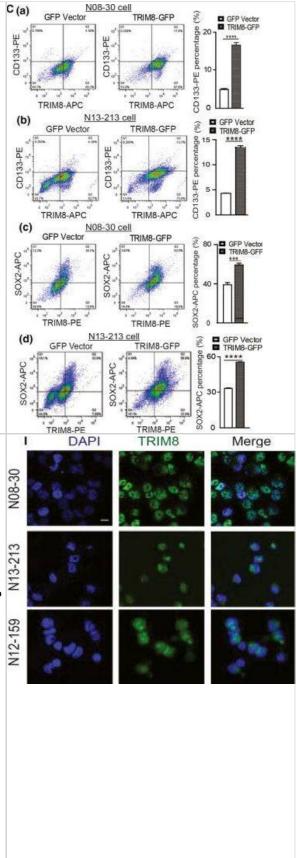


Flow Cytometry: TRIM8 Antibody [NBP1-89776] - Knockdown of TRIM8 promotes differentiation & impairs GBM stemness & self □renewal capacity. (A) Western blot analysis shows reduced CD133, NESTIN, SOX2, & c□MYC, & upregulated GFAP in GBM neurosphere cells following TRIM8 knockdown by TRIM8 shRNA1 or TRIM8 shRNA2. NT shRNA = nontargeting control shRNA. Western blot also shows upregulated PIAS3 & reduced phosphorylated STAT3 (Tyr705) following shRNA knockdown of TRIM8. (B) ICC shows reduced expression of TRIM8 (red), NESTIN (white), & SOX2 (red) in TRIM8 shRNA1 knockdown cells compared to NT shRNA cells. Nuclei were counterstained with DAPI (blue). Scale bar = 10 µm. (C) Flow cytometric analysis & statistical analysis of SOX2 expression in TRIM8 □ overexpressing neurosphere cells. SOX2 was tagged by APC (blue: 676 nm) with TRIM8 tagged by PE (red: 555 nm). \*\*\*P < 0.001, \*\*\*\*P < 0.0001. Statistics: Data are means  $\pm$  SD (n = 3), by nonparametric t test. (D,E) TRIM8 knockdown impairs neurosphere formation. Representative images of neurosphere expressing NT shRNA & TRIM8 shRNA1 at specific time points are shown. Scale bar = 30 µm. Statistical analysis of average sphere size: by one way analysis of variance (ANOVA). Data are means ± standard deviation (SD) (n = 6). \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001. (F) Western blot analysis shows reduced TRIM8 & activated STAT3 (Tyr705), with increased GFAP expression following differentiation induced by serum. (G) ICC reveals reduced TRIM8 (red) & increased GFAP (white) after four days of differentiation induced by serum. Nuclei were counterstained with DAPI (blue). Scale bar = 10 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28100038), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

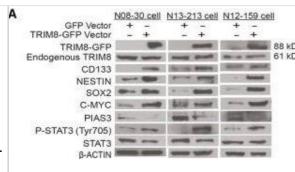


Flow Cytometry: TRIM8 Antibody [NBP1-89776] - Ectopic expression of TRIM8 enhances stemness & self renewal of GBM derived neurospheres. (A) Western blot analysis shows upregulation of CD133, NESTIN, SOX2, & c□MYC following ectopic expression of TRIM8□GFP fusion protein. WB also shows reduced PIAS3 & increased phosphorylated STAT3 (Tyr705) & total STAT3 following TRIM8 overexpression. (B) ICC staining of TRIM8 (red) with stem cell markers NESTIN (white), & SOX2 (red) in neurosphere cells overexpressing TRIM8. Nuclei were counterstained with DAPI (blue). Scale bar = 10 µm. (C) Flow cytometric analysis & statistical analysis showing CD133 & SOX2 upregulation in neurosphere cells with TRIM8 overexpression. CD133 was tagged by PE (red: 555 nm) with TRIM8 tagged by APC (blue: 676 nm). SOX2 was tagged by APC (blue: 676 nm) with TRIM8 tagged by PE (red: 555 nm). \*\*\*P < 0.001, \*\*\*\*P < 0.0001. Statistics: Data are means  $\pm$  SD (n = 3), by nonparametric t $\square$ test. (D,E) Effects of TRIM8 overexpression on neurosphere formation. Representative images of neurospheres expressing GFP vector or TRIM8□GFP vector at specific points. Scale bar = 30 µm. Statistical analysis of average sphere size: by one □way analysis of variance (ANOVA). Data are means  $\pm$  standard deviation (SD) (n = 6). \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001. (F) Extreme limiting dilution assay (ELDA) shows that TRIM8 overexpression reduces the cell dose required for colony formation, supporting enhanced stem cell properties. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28100038), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

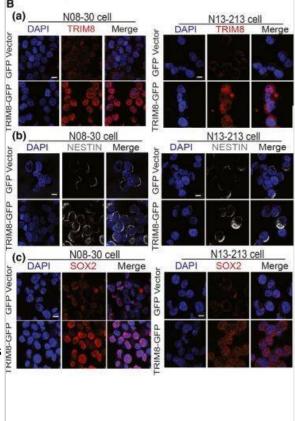
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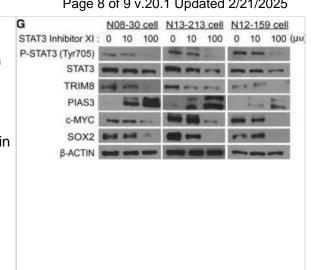
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#### **Publications**

Zhang C, Mukherjee S, Tucker-Burden C et al. TRIM8 regulates stemness in glioblastoma through PIAS3-STAT3 Mol Oncol 2017-02-15 [PMID: 28100038] (WB, FLOW, Human)





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techne.com

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General: novus@novusbio.com

## **Products Related to NBP1-89776**

NBP1-89776PEP TRIM8 Recombinant Protein Antigen

HAF008 Goat anti-Rabbit IgG Secondary Antibody [HRP]

NB7160 Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]

NBP2-24891 Rabbit IgG Isotype Control

#### 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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