# **Product Datasheet**

# FOXO3 Antibody - BSA Free NBP2-16521

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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#### NBP2-16521

FOXO3 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	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.025% Proclin 300
Isotype	IgG
Purity	Antigen Affinity-purified
Buffer	PBS, 20% Glycerol
Target Molecular Weight	71 kDa

Product Description	
Description	Novus Biologicals Rabbit FOXO3 Antibody - BSA Free (NBP2-16521) is a polyclonal antibody validated for use in IHC, WB, ICC/IF, IP and ChIP. Anti-FOXO3 Antibody: Cited in 12 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	2309
Gene Symbol	FOXO3
Species	Human, Mouse, Rat, Squirrel, Xenopus
Immunogen	Carrier-protein conjugated synthetic peptide encompassing a sequence within the C-terminus region of human FOXO3. The exact sequence is proprietary.

<b>Product Application Details</b>	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Knockdown Validated
Recommended Dilutions	Western Blot 1:500-1:3000, Immunohistochemistry 1:100-1:1000, Immunocytochemistry/ Immunofluorescence 1:100-1:1000, Immunoprecipitation 1:100-1:500, Immunohistochemistry-Paraffin 1:100-1:1000, Chromatin Immunoprecipitation (ChIP) Reported in scientific literature (PMID: 26229077), Knockdown Validated

# **Images**

Western Blot: FOXO3 Antibody [NBP2-16521] - FoxO3 expression in nuclear and cytosolic fraction of human breast cancer cell line MDA-MB-231. Image from verified customer review.

FoxO3

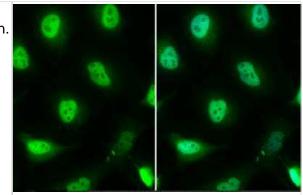


Nuclea

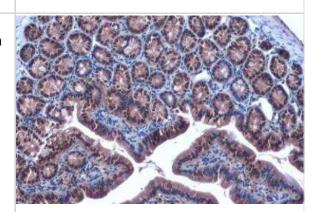
Cytosolic



Immunocytochemistry/Immunofluorescence: FOXO3 Antibody [NBP2-16521] - HeLa cells were fixed in 4% paraformaldehyde at RT for 15 min. Green: FOXO3A stained by FOXO3A antibody [C3], C-term diluted at 1:1000. Blue: Hoechst 33342 staining.

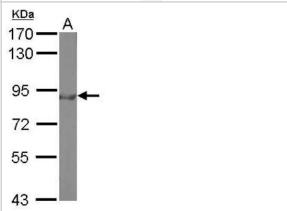


Immunohistochemistry-Paraffin: FOXO3 Antibody [NBP2-16521] - Mouse duodenum. FOXO3A stained by FOXO3A antibody [C3], C-term diluted at 1:2000. Antigen Retrieval: Citrate buffer, pH 6.0, 15 min.

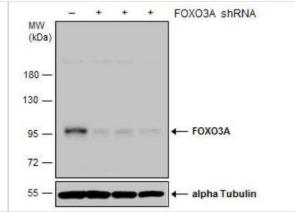


Chromatin Immunoprecipitation (ChIP): FOXO3 Antibody [NBP2-16521] - (D) ChIP assay for LMOD1 and EGFR (as a positive control region) in HCASMC chromatin lysates immunoprecipitated with antibodies to FOXO3 or a negative control rabbit IgG. n = 3. (E) Allele specific ChIP (haploChIP) for FOXO3 protein in DNA derived from cultured HCASMC ChIP experiments. DNA from cell line homozygous for the ancestral allele was used as a positive control and arbitrarily set to 1. Values represent mean +/- standard deviation of triplicates. Similar results were observed from n = 4 independent lines for each genotype. (F) Quantitative RT-PCR analysis of LMOD1 in HCASMCs following knock down of endogenous FOXO3 (F) or overexpression of FOXO3 (G). The results were reproduced in n = 3 independent experiments. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pgen.1007755) licensed under a CC-BY license.

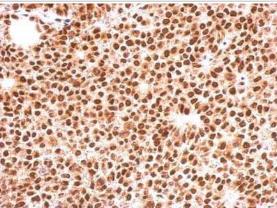
Western Blot: FOXO3 Antibody [NBP2-16521] - 50 ug of mouse brain whole cell lysate). Antibody at 1:500.



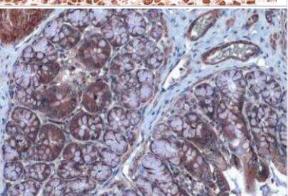
Western Blot: FOXO3 Antibody [NBP2-16521] - Non-transfected (-) and transfected (+) C2C12 whole cell extracts (30 ug) were separated by 7.5% SDS-PAGE, and the membrane was blotted with FOXO3A antibody [C3], C-term.



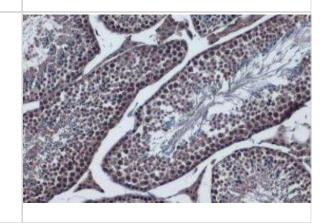
Immunohistochemistry-Paraffin: FOXO3 Antibody [NBP2-16521] - Huh-7 xenograft, using FOXO3A antibody at 1:500 dilution. Antigen Retrieval: Trilogy™ (EDTA based, pH 8.0) buffer, 15min.



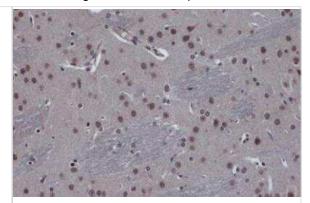
Immunohistochemistry-Paraffin: FOXO3 Antibody [NBP2-16521] - Rat colon. FOXO3A stained by FOXO3A antibody [C3], C-term diluted at 1:2000. Antigen Retrieval: Citrate buffer, pH 6.0, 15 min.



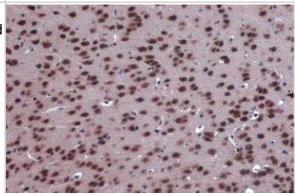
Immunohistochemistry-Paraffin: FOXO3 Antibody [NBP2-16521] - Mouse testis. FOXO3A stained by FOXO3A antibody [C3], C-term diluted at 1:2000. Antigen Retrieval: Citrate buffer, pH 6.0, 15 min.



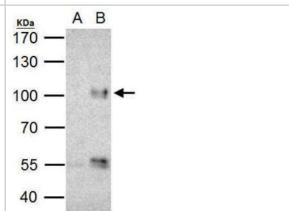
Immunohistochemistry-Paraffin: FOXO3 Antibody [NBP2-16521] - Rat brain. FOXO3A stained by FOXO3A antibody [C3], C-term diluted at 1:2000. Antigen Retrieval: Citrate buffer, pH 6.0, 15 min.



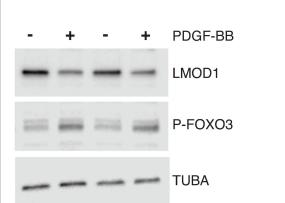
Immunohistochemistry-Paraffin: FOXO3 Antibody [NBP2-16521] - Mouse brain. FOXO3A stained by FOXO3A antibody [C3], C-term diluted at 1:2000. Antigen Retrieval: Citrate buffer, pH 6.0, 15 minutes.



Immunoprecipitation: FOXO3 Antibody [NBP2-16521] - Jurkat whole cell lysate/extract A. Control with 2 ug of preimmune rabbit IgG B. Immunoprecipitation of FOXO3A protein by 2 ug of FOXO3A antibody 5% SDS-PAGE The immunoprecipitated FOXO3A protein was detected by FOXO3A antibody diluted at 1:1000. EasyBlot anti-rabbit IgG was used as a secondary reagent.

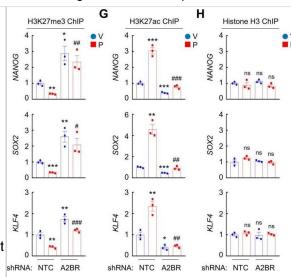


Western Blot: FOXO3 Antibody [NBP2-16521] - LMOD1 expression is mediated by PDGF-BB-FOXO3 signaling cascade.(A) UCSC Browser screenshot of the LMOD1 CAD locus at chromosome 1q32.1 highlighting the candidate causal variant, rs34091558, overlapping ATAC-seq open chromatin & RNA-seq tracks in HCASMCs treated with PDGF-BB (n = 2 biological replicates). Genomic coordinates refer to hg19 assembly. Quantitative RT-PCR (B) & Western blotting (C) data revealing PDGF-BB & phosphorylated-FOXO3 (P-FOXO3) mediated LMOD1 expression. (D) Co-expression microarray analysis performed in a cohort of carotid atherosclerotic plaques (n = 127) indicating that LMOD1 & FOXO3 are positively correlated in arterial tissues. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30444878), licensed under a CC-BY

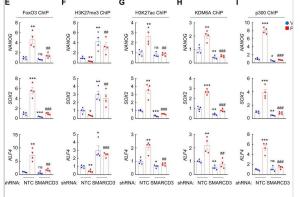


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Immunoprecipitation: FOXO3 Antibody [NBP2-16521] - Chemotherapyinduced A2BR expression promotes FOXO3 binding on pluripotency factor genes through decreased H3K27me3 & increased H3K27ac chromatin marks. A & B, MDA-MB-231 NTC or A2BR knockdown subclones were transfected with pLX304 (empty vector, EV) or pLX304 encoding A2BR. Cells were treated with vehicle (V) or 10 nM paclitaxel (P) for 72 h & chromatin immunoprecipitation (ChIP) was performed with antibody against FOXO3. Primers flanking the FOXO3 binding site in the NANOG, SOX2 & KLF4 gene (A) were used for qPCR (B; mean ± SEM; n = 3); \*\*p < 0.01, \*\*\*p < 0.001 vs. NTC/EV-V; #p < 0.05, ##p < 0.01 vs. NTC/EV-P; Mp < 0.01, Mp < 0.0001 vs. A2BR shRNA/EV-P; ns, not significant. C & E, MDA-MB-231 NTC or A2BR knockdown subclones were treated with V or P for 72 h & immunoblot assays were performed. D. MDA-MB-231 NTC or A2BR knockdown subclones were treated with V or P for 72 h. Cytosolic & nuclear lysates were prepared, & immunoblot assays were performed. F-H, MDA-MB-231 NTC or A2BR knockdown subclones were treated with V or 10 nM P for 72 h. ChIP was performed using antibodies against H3K27me3 (F), H3K27ac (G), or Histone H3 (H) followed by qPCR with primers flanking FOXO3 binding sites in the NANOG, SOX2 & KLF4 gene (mean  $\pm$  SEM; n = 3); \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 vs. NTC-V; #p < 0.05, ##p < 0.01, ###p < 0.001 vs. NTC-P; ns, not significant. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35401817), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



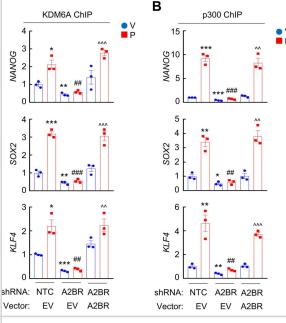
Immunoprecipitation: FOXO3 Antibody [NBP2-16521] - SMARCD3 knockdown blocks paclitaxel-induced FOXO3 binding on pluripotency factor genes & inhibits BCSC enrichment. A, MDA-MB-231 cells were transfected with vector encoding NTC or either of two shRNAs targeting SMARCD3 (#1 & #2), & immunoblot assay was performed. B-D, MDA-MB-231 NTC or SMARCD3 knockdown subclones were treated with vehicle (V) or 10 nM paclitaxel (P) for 72 h, & ALDH (B; mean ± SEM; n = 3), mammosphere (C; mean ± SEM; n = 4), & qPCR (D; mean ± SEM; n = 3) assays were performed; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 vs. NTC-V; #p < 0.05, #p < 0.01, #p < 0.001 vs. NTC-P; ns. not significant. E-I, MDA-MB-231 NTC or SMARCD3 knockdown subclones were treated with V or 10 nM P for 72 h. ChIP was performed using antibodies against FOXO3 (E), H3K27me3 (F), H3K27ac (G), KDM6A (H), or p300 (I), followed by qPCR with primers flanking FOXO3 binding sites in the NANOG, SOX2 & KLF4 gene (mean ± SEM; n = 4); \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 vs. NTC-V; ##p < 0.01, ###p < 0.001 vs. NTC-P; ns, not significant. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35401817), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



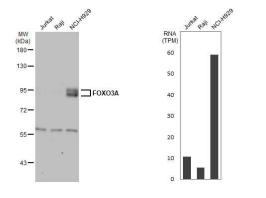
Immunoprecipitation: FOXO3 Antibody [NBP2-16521] - Chemotherapyinduced A2BR expression promotes FOXO3 binding on pluripotency factor genes through decreased H3K27me3 & increased H3K27ac chromatin marks. A & B, MDA-MB-231 NTC or A2BR knockdown subclones were transfected with pLX304 (empty vector, EV) or pLX304 encoding A2BR. Cells were treated with vehicle (V) or 10 nM paclitaxel (P) for 72 h & chromatin immunoprecipitation (ChIP) was performed with antibody against FOXO3. Primers flanking the FOXO3 binding site in the NANOG, SOX2 & KLF4 gene (A) were used for qPCR (B; mean ± SEM; n = 3); \*\*p < 0.01, \*\*\*p < 0.001 vs. NTC/EV-V; #p < 0.05, ##p < 0.01 vs. NTC/EV-P;  $^p$  < 0.01,  $^p$  < 0.0001 vs. A2BR shRNA/EV-P; ns, not significant. C & E, MDA-MB-231 NTC or A2BR knockdown subclones were treated with V or P for 72 h & immunoblot assays were performed. D. MDA-MB-231 NTC or A2BR knockdown subclones were treated with V or P for 72 h. Cytosolic & nuclear lysates were prepared, & immunoblot assays were performed. F-H, MDA-MB-231 NTC or A2BR knockdown subclones were treated with V or 10 nM P for 72 h. ChIP was performed using antibodies against H3K27me3 (F), H3K27ac (G), or Histone H3 (H) followed by qPCR with primers flanking FOXO3 binding sites in the NANOG, SOX2 & KLF4 gene (mean ± SEM; n = 3); \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 vs. NTC-V; #p < 0.05, ##p < 0.01, ###p < 0.001 vs. NTC-P; ns, not significant. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35401817), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

A В FoxO3 ChIP 10 -3.6 kb NANOG 6 NANOG 4 CTATT**TGTTTAC**AAAAC 10 -4.6 kb 8 6 SOX2 SOX2 **GCACTTATTTACCTTCA** 0 -1.7 kb 6 KLF4 KLF4 CGAGGTGTTTACTTTGG shRNA: NTC A2BR A2BR Vector: FV EV A2BR

Immunoprecipitation: FOXO3 Antibody [NBP2-16521] - A2BR decreases H3K27me3 & increases H3K27ac marks through recruitment of KDM6A & p300 at FOXO3 binding sites of pluripotency factor genes. A & B, MDA-MB-231 NTC or A2BR knockdown subclones were transfected with pLX304 (empty vector, EV) or pLX304 encoding A2BR. Cells were treated with vehicle (V) or 10 nM paclitaxel (P) for 72 h. ChIP was performed using antibodies against KDM6A (A) or p300 (B) followed by qPCR with primers flanking FOXO3 binding sites in the NANOG, SOX2 & KLF4 gene (mean  $\pm$  SEM; n = 3); \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 vs. NTC-V; ##p < 0.01, ###p < 0.001 vs. NTC-P;  $^p$  < 0.01,  $^p$  < 0.0001 vs. A2BR shRNA/EV-P; ns, not significant. C, MDA-MB-231 NTC or A2BR knockdown subclones were treated with V or P for 72 h & immunoblot assays were performed. D, MDA-MB-231 NTC or A2BR knockdown subclones were treated with V or P for 72 h & nuclear lysates were prepared. Immunoprecipitation (IP) was performed using FOXO3 antibody or control IgG followed by immunoblot assays. NL, nuclear protein lysate. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35401817), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: FOXO3 Antibody [NBP2-16521] - Various whole cell extracts (30 ug) were separated by 7.5% SDS-PAGE, and the membrane was blotted with FOXO3A antibody [C3], C-term diluted at 1:1000. The HRP-conjugated anti-rabbit IgG antibody was used to detect the primary antibody. Corresponding RNA expression data for the same cell lines are based on Human Protein Atlas program.





#### **Publications**

Park M, Baker W, Cambow D et al. Methamphetamine Enhances HIV-Induced Aberrant Proliferation of Neural Progenitor Cells via the FOXO3-Mediated Mechanism Molecular Neurobiology 2021-11-01 [PMID: 33983546] (Western Blot, Mouse)

Yan D, He Q, Pei L et al. The APC/C E3 ligase subunit ANAPC11 mediates FOXO3 protein degradation to promote cell proliferation and lymph node metastasis in urothelial bladder cancer Cell Death & Disease 2023-08-12 [PMID: 37573356] (Western Blot, Mouse)

David Romeo-Guitart, César Marcos-DeJuana, Sara Marmolejo-Martínez-Artesero, Xavier Navarro, Caty Casas Novel neuroprotective therapy with NeuroHeal by autophagy induction for damaged neonatal motoneurons Theranostics 2020-01-01 [PMID: 32308774]

Qiu CW, Chen B, Zhu HF et al. Gastrodin alleviates cisplatin nephrotoxicity by inhibiting ferroptosis via the SIRT1/FOXO3A/GPX4 signaling pathway Journal of ethnopharmacology 2023-10-05 [PMID: 37802374] (Immunoprecipitation)

Xing X, Liu M, Wang X et al. Promoting effects of calponin 3 on the growth of diffuse large B?cell lymphoma cells Oncology reports 2023-02-01 [PMID: 36660952] (ChIP, Human)

Niu J, Yan T, Guo W et al. The COPS3-FOXO3 positive feedback loop regulates autophagy to promote cisplatin resistance in osteosarcoma Autophagy 2022-11-30 [PMID: 36451342] (Chemotaxis, Human)

Lan J, Wei G, Liu J et al. Chemotherapy-induced adenosine A2B receptor expression mediates epigenetic regulation of pluripotency factors and promotes breast cancer stemness Theranostics 2022-02-28 [PMID: 35401817] (IP, Human)

Wang JJ, Ge W, Zhai QY et al. Single-cell transcriptome landscape of ovarian cells during primordial follicle assembly in mice PLoS biology 2020-12-22 [PMID: 33351795] (IF/IHC, Mouse)

Yan, S;Wang, Q;Huo, Z;Yang, T;Yin, X;Wang, Z;Zhang, Z;Wu, H; Gene expression profiles between cystic and solid vestibular schwannoma indicate susceptible molecules and pathways in the cystic formation of vestibular schwannoma Funct. Integr. Genomics 2019-04-05 [PMID: 30953268] (IF/IHC, Human)

Nanda V, Wang T, Pjanic M et al. Functional regulatory mechanism of smooth muscle cell-restricted LMOD1 coronary artery disease locus. PLoS Genet. 2018-11-16 [PMID: 30444878] (Chemotaxis, Human)

Lu H Samanta D Xiang L et al. Chemotherapy triggers HIF-1-dependent glutathione synthesis and copper chelation that induces the breast cancer stem cell phenotype. Proc Natl Acad Sci U S A. 2015-07-30 [PMID: 26229077] (Chemotaxis, WB, Human)

Sinanoglu O, Yener AN, Ekici S et al. The protective effects of spirulina in cyclophosphamide induced nephrotoxicity and urotoxicity in rats. Urology 2012-12-01 [PMID: 22951000] (WB, Mouse)





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### **Products Related to NBP2-16521**

HAF008 Goat anti-Rabbit IgG Secondary Antibody [HRP]

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

NBP2-24891 Rabbit IgG Isotype Control

H00002309-Q01-10ug Recombinant Human FOXO3 GST (N-Term) Protein

#### 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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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