

# Product Datasheet

## Serpin A8/Angiotensinogen Antibody - BSA Free NBP1-30027

Unit Size: 0.2 ml

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

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[technical@novusbio.com](mailto:technical@novusbio.com)

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**NBP1-30027**

Serpine A8/Angiotensinogen Antibody - BSA Free

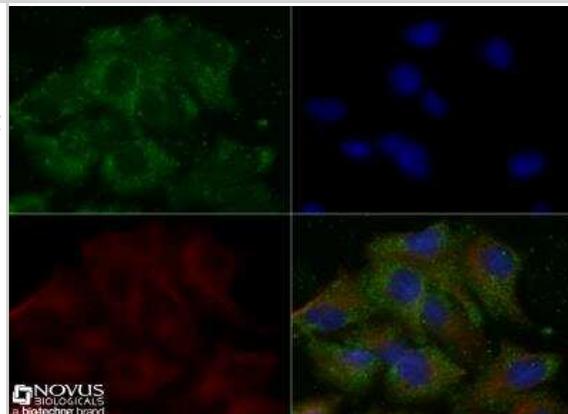
<b>Product Information</b>	
<b>Unit Size</b>	0.2 ml
<b>Concentration</b>	1 mg/ml
<b>Storage</b>	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
<b>Clonality</b>	Polyclonal
<b>Preservative</b>	0.02% Sodium Azide
<b>Isotype</b>	IgG
<b>Purity</b>	Immunogen affinity purified
<b>Buffer</b>	PBS
<b>Target Molecular Weight</b>	53 kDa
<b>Product Description</b>	
<b>Host</b>	Rabbit
<b>Gene ID</b>	183
<b>Gene Symbol</b>	AGT
<b>Species</b>	Human, Mouse, Rat
<b>Immunogen</b>	A synthetic peptide made to an N-terminal portion of the human Angiotensinogen protein (between residues 1-50) [UniProt P01019]
<b>Product Application Details</b>	
<b>Applications</b>	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
<b>Recommended Dilutions</b>	Western Blot 1:1000, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:200, Immunohistochemistry-Paraffin 1:200
<b>Application Notes</b>	This Angiotensinogen antibody is useful for Western Blot, Immunocytochemistry/Immunofluorescence, and Immunohistochemistry paraffin embedded sections. In Western Blot, a band is seen ~53 kDa representing Angiotensinogen. In ICC/IF, cytoplasmic staining was observed in HepG2 cells. In IHC-P, secreted and cytoplasmic staining was observed in mouse kidney tissue. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

## Images

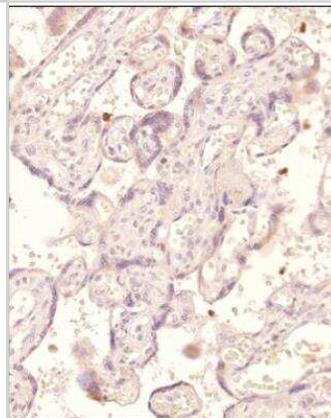
Western Blot: Serpin A8/Angiotensinogen Antibody [NBP1-30027] - Analysis of Angiotensinogen in human kidney lysate.

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<25  
<20  
<15  
<10

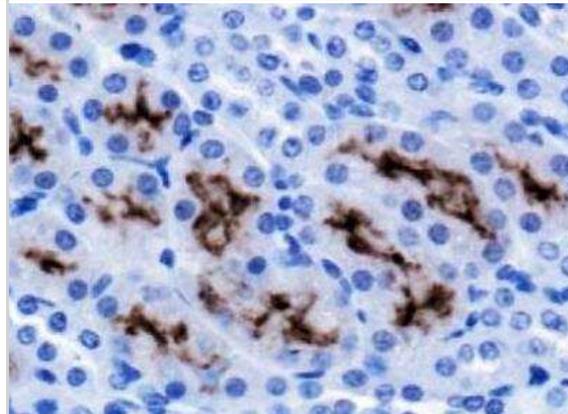
Immunocytochemistry/Immunofluorescence: Serpin A8/Angiotensinogen Antibody [NBP1-30027] - HepG2 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-Angiotensinogen at 10 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Immunohistochemistry-Paraffin: Serpin A8/Angiotensinogen Antibody - BSA Free [NBP1-30027] - Analysis of a FFPE tissue section of human placenta using 1:200 dilution of Serpin A8 (NBP1-30027) antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



Immunohistochemistry-Paraffin: Serpin A8/Angiotensinogen Antibody [NBP1-30027] - Analysis of Angiotensinogen in mouse kidney using DAB with hematoxylin counterstain.



## Publications

Marry A S Cirilo, Valéria B S Santos, Natália K S Lima, Humberto Muzi-Filho, Ana D O Paixão, Adalberto Vieyra, Leucio D Vieira Reactive oxygen species impair Na<sup>+</sup> transport and renal components of the renin-angiotensin-aldosterone system after paraquat poisoning. *Anais da Academia Brasileira de Ciencias* 2024-04-11 [PMID: 38597493]

Liu J, Li Y, Zhang Y et al. Single cell RNA-seq analysis identifies angiotensinogen and galanin as unique molecular markers of acinar cells in murine salivary glands *Stem cells and development* 2023-10-12 [PMID: 37823745]

Lima NKS, Farias WRA, Cirilo MAS et al. Renal ischemia-reperfusion leads to hypertension and changes in proximal tubule Na<sup>+</sup> transport and renin-angiotensin-aldosterone system: Role of NADPH oxidase *Life sciences* 2020-12-10 [PMID: 33310030] (WB, Rat)

Mota GAF, de Souza SLB, da Silva VL et al. Cardioprotection Generated by Aerobic Exercise Training is Not Related to the Proliferation of Cardiomyocytes and Angiotensin-(1-7) Levels in the Hearts of Rats with Supravalvar Aortic Stenosis *Cell. Physiol. Biochem.* 2020-07-31 [PMID: 32730701] (WB, Rat)

Sukketsiri W, Hoshino K, Kugo H et al. Isoflavone Ameliorated Oxidative Stress and Vascular Damages in Nicotine-Administrated Mice *J Oleo Sci* 2019-12-01 [PMID: 31735744] (IHC-P, Mouse)

Kim YJ, Kim Yn, Kang B- et al. Induction of multiple ovulation via modulation of angiotensin II receptors in in vitro ovarian follicle culture models *J Tissue Eng Regen Med* 2016-09-15 [PMID: 27717202] (WB, Mouse)

Wu Y, Ma KL, Zhang Y et al. Lipid disorder and intrahepatic renin-angiotensin system activation synergistically contributes to non-alcoholic fatty liver disease. *Liver Int.* 2016-03-30 [PMID: 27028410] (WB, Human)

Ishigami T, Kino T, Chen L et al. Identification of Bona Fide Alternative Renin Transcripts Expressed Along Cortical Tubules and Potential Roles in Promoting Insulin Resistance In Vivo Without Significant Plasma Renin Activity Elevation. *Hypertension.* 2014-04-28 [PMID: 24777979] (IHC-P, Mouse)

Ahnstedt H, Cao L, Krause DN et al. Male-female differences in upregulation of vasoconstrictor responses in Human cerebral arteries. *PLoS One* 2013-04-29 [PMID: 23658641] (IF/IHC, ICC/IF, Human)

Shababi M, Habibi J, Ma L, Glascock JJ, Sowers JR, Lorson CL. Partial restoration of cardio-vascular defects in a rescued severe model of spinal muscular atrophy. *J Mol Cell Cardiol*;52(5):1074-82. 2012-05-01 [PMID: 22285962] (WB, Mouse)

Dimitrijevic I, Rissler P, Luts L, Edvinsson L. Reduced expression of angiotensin II and angiotensin receptor type 1 and type 2 in resistance arteries from nasal lesions in granulomatosis with polyangiitis (Wegener's granulomatosis). *Scand J Rheumatol*;40(6):448-52. 2011-11-01 [PMID: 21936613] (IF/IHC, ICC/IF, Human)



## Procedures

### Western Blot Protocol specific for Angiotensin antibody (NBP1-30027)

Serpin A8/Angiotensinogen Antibody:

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer 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 manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

\*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

### Immunohistochemistry-Paraffin Embedded Sections Protocol specific for Angiotensin antibody (NBP1-30027)

Serpin A8/Angiotensinogen Antibody:

Immunohistochemistry-Paraffin Embedded Sections Protocol

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.

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution 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 biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

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**Immunocytochemistry/Immunofluorescence Protocol for Angiotensinogen Antibody (NBP1-30027)**

Serpine A8/Angiotensinogen Antibody:

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

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### **Novus Biologicals USA**

10730 E. Briarwood Avenue  
Centennial, CO 80112  
USA  
Phone: 303.730.1950  
Toll Free: 1.888.506.6887  
Fax: 303.730.1966  
nb-customerservice@bio-techne.com

### **Bio-Techne Canada**

21 Canmotor Ave  
Toronto, ON M8Z 4E6  
Canada  
Phone: 905.827.6400  
Toll Free: 855.668.8722  
Fax: 905.827.6402  
canada.inquires@bio-techne.com

### **Bio-Techne Ltd**

19 Barton Lane  
Abingdon Science Park  
Abingdon, OX14 3NB, United Kingdom  
Phone: (44) (0) 1235 529449  
Free Phone: 0800 37 34 15  
Fax: (44) (0) 1235 533420  
info.EMEA@bio-techne.com

### **General Contact Information**

www.novusbio.com  
Technical Support: nb-technical@bio-techne.com  
Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

### **Products Related to NBP1-30027**

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HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NBP1-30027B	Serpin A8/Angiotensinogen Antibody [Biotin]

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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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