



PRODUCT INFORMATION & ELISA MANUAL

Serpin A8/Angiotensinogen Antibody Pair [HRP]

NBP2-79330

Sample Insert for reference use only

Matched Antibody Pair utilized in an Enzyme-linked
Immunosorbent Assay for quantitative detection of
Human Serpin A8/Angiotensinogen.

For research use only.

Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

BACKGROUND

Angiotensinogen, also known as AGT and SerpinA8, is a member of the serpin family. It is an α -2-globulin that is produced constitutively and released into the circulation mainly by the liver. Angiotensinogen is an essential component of the renin-angiotensin system (RAS) and a potent regulator of blood pressure. Angiotensinogen can be schematically considered to consist of a combination of an angiotensin I (Ang I) function, located at the N-terminal end, and the presence of a serpin (serine protease inhibitor) structure at the opposite end. Angiotensinogen is cleaved into three chains: Angiotensin-1 (Ang I), Angiotensin-2 (Ang II), and Angiotensin-3 (Ang III). Angiotensin-1 is a substrate of ACE (angiotensin converting enzyme) that removes a dipeptide to yield the physiologically active peptide angiotensin-2. Angiotensin-1 and angiotensin-2 can be further processed to generate angiotensin-3, angiotensin-4. Angiotensin 1-7 is cleaved from angiotensin-2 by ACE2. Angiotensin-2 acts directly on vascular smooth muscle as a potent vasoconstrictor, affects cardiac contractility and heart rate through its action on the sympathetic nervous system. Defects in AGT are associated with susceptibility to essential hypertension and renal tubular dysgenesis (RTD). Several serpins (antithrombin, maspin, pigment epithelial-derived factor, and kallistatin) have been recently shown to exert an antiangiogenic activity, suggesting a common mechanism of endothelial cell proliferation and migration. Angiotensinogen/AGT and its renin-cleaved product, des(Ang I)AGT, are also angiogenesis inhibitors, both in vitro and in vivo at concentrations within the range of those observed in plasma. The Angiotensinogen products, that is angiotensin II and possibly angiotensin II-related products, have been found to act locally in modulating adipose tissue growth in an autocrine/paracrine manner. The transient or chronic overexpression of angiotensinogen in adipose tissue favors lipogenesis in adipocytes and leads to a 'vicious' circle whereby adipose tissue development is further increased.

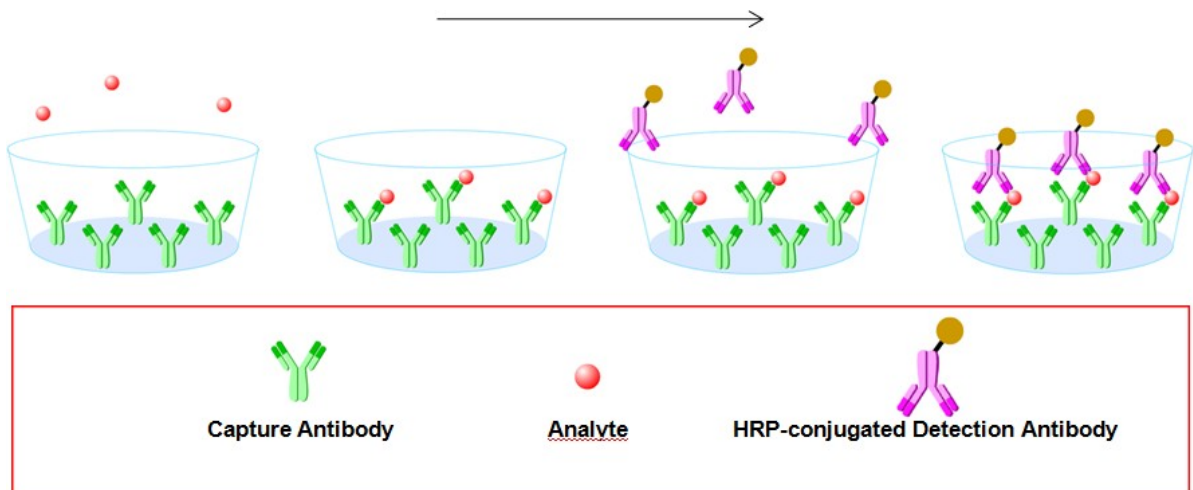
PRINCIPLE OF THE TEST

The Novus Biologicals Serpin A8/Angiotensinogen Antibody Pair [HRP] is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for Serpin A8/Angiotensinogen coated on a 96- well plate. Standards and samples are added to the wells, and any Serpin A8/Angiotensinogen present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated rabbit anti-Serpin A8/Angiotensinogen polyclonal antibody is then added, producing an antibody-antigen-antibody “sandwich”. The wells are again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of Serpin A8/Angiotensinogen present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

INTENDED USE

- ◆ The Serpin A8/Angiotensinogen Antibody Pair [HRP] is for the quantitative determination of Human Serpin A8/Angiotensinogen.
- ◆ This Serpin A8/Angiotensinogen Antibody Pair [HRP] contains the basic components required for the development of sandwich ELISAs.

ASSAY PROCEDURE SUMMARY



This antibody pair has been configured for research use only and is not to be used in diagnostic procedures.

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody – 0.25 mg/mL of mouse anti-Serpin A8/Angiotensinogen monoclonal antibody. Dilute to a working concentration of 1.0 µg/mL in CBS before coating.

Detection Antibody – 0.5 mg/mL rabbit anti-Serpin A8/Angiotensinogen polyclonal antibody conjugated to horseradish-peroxidase (HRP). Dilute to working concentration of 0.5 µg/mL in detection antibody dilution buffer before use.

Standard – Each vial contains 550 ng of recombinant Serpin A8/Angiotensinogen. Reconstitute standard powder with 1 mL detection antibody dilution buffer. After reconstitution, store at -20°C to -80°C in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 15000 pg/mL is recommended.

SOLUTIONS REQUIRED

CBS - 0.05M Na₂CO₃, 0.05M NaHCO₃, pH 9.6, 0.2 µm filtered

TBS - 20 mM Tris, 150 mM NaCl, pH 7.4

Wash Buffer - 0.05% Tween20 in TBS, pH 7.2 - 7.4

Blocking Buffer - 2% BSA in Wash Buffer

Sample dilution buffer - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

Detection antibody dilution buffer - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

Substrate Solution : To achieve best assay results, fresh substrate solution is recommended

Substrate stock solution - 10mg / ml TMB (Tetramethylbenzidine) in DMSO

Substrate dilution buffer - 0.05M Na₂HPO₄ and 0.025M citric acid ; adjust pH to 5.5

Substrate working solution - For each plate dilute 250 µl substrate stock solution in 25ml substrate dilution buffer and then add 80 µl 0.75% H₂O₂, mix it well

Stop Solution - 2 N H₂SO₄

PRECAUTION

The Stop Solution suggested for use with this antibody pair is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

STORAGE

Capture Antibody: Aliquot and store at -20°C to -80°C for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Detection Antibody: Protect it from prolonged exposure to light. Aliquot and store at -20°C to -80°C and for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Standard: Store lyophilized standard at -20°C to -80°C for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at -80°C for up to 1 month. Avoid repeated freeze-thaw cycles.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100µL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4°C.
2. Aspirate each well and wash with at least 300µl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

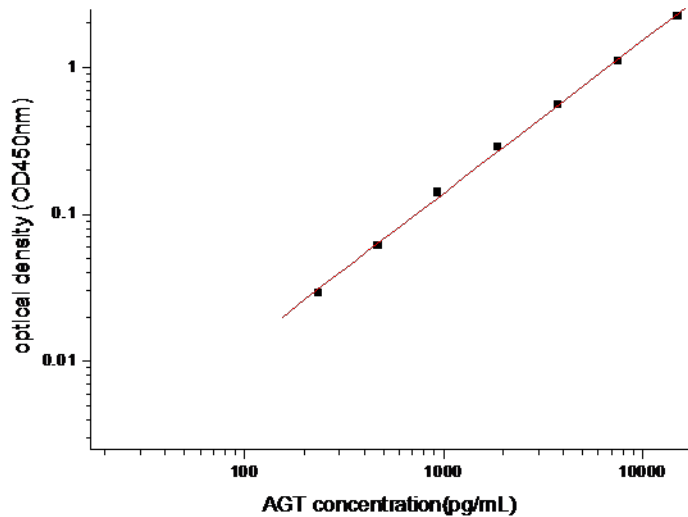
1. Add 100 µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200 µL of substrate solution to each well. Incubate for 20 minutes at room temperature (**if substrate solution is not as requested, the incubation time should be optimized**). Avoid placing the plate in direct light.
6. Add 50 µL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
- Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
- To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.



Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
234.375	0.029
468.75	0.061
937.5	0.141
1875	0.286
3750	0.561
7500	1.100
15000	2.216

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of Human Serpin A8/Angiotensinogen was determined to be approximately 234.375 pg/ml. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 °C
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated.	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	Try higher dilution rate of samples	

ELISA Plate Template

[illegible]

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Notes