



PRODUCT INFORMATION & ELISA MANUAL

Serpin A1/alpha 1-Antitrypsin Antibody Pair [HRP]

NBP2-79565

Sample Insert for reference use only

Matched Antibody Pair utilized in an Enzyme-linked
Immunosorbent Assay for quantitative detection of
Human Serpin A1/alpha 1-Antitrypsin.

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

SerpinA1, also known as Alpha-1 antitrypsin (AAT), is a prototype member of the Serpin superfamily of the serine protease inhibitors. This serine protease inhibitor blocks the protease, neutrophil elastase. Alpha-1 antitrypsin is mainly produced in the liver and acts as an antiprotease. Its principal function is to inactivate neutrophil elastase, preventing tissue damage. SerpinA1 (alpha1-antitrypsin), an acute phase protein and the classical neutrophil elastase inhibitor, is localized within lipid rafts in primary human monocytes in vitro. Its association with monocytes is inhibited by cholesterol depleting/efflux-stimulating agents (nystatin, filipin, MbetaCD (methyl-beta-cyclodextrin) and oxidized low-density lipoprotein (oxLDL) and conversely, enhanced by free cholesterol. Furthermore, SerpinA1/monocyte association per se depletes lipid raft cholesterol as characterized by the activation of extracellular signal-regulated kinase 2, formation of cytosolic lipid droplets, and a complete inhibition of oxLDL uptake by monocytes. Previous population studies have suggested that heterozygote status for the AAT gene (SerpinA1) is a risk factor for chronic rhinosinusitis with nasal polyposis (CRSwNP). Alpha-1 antitrypsin deficiency is a recently identified genetic disease that occurs almost as frequently as cystic fibrosis. It is caused by various mutations in the SerpinA1 gene, and has numerous clinical implications. Alpha-1 antitrypsin deficiency is an inherited disease affecting the lung and liver. In the liver, alpha-1 antitrypsin deficiency may manifest as benign neonatal hepatitis syndrome; a small percentage of adults develop liver fibrosis, with progression to cirrhosis and hepatocellular carcinoma. Its most important physiologic functions are the protection of pulmonary tissue from aggressive proteolytic enzymes and regulation of pulmonary immune processes.

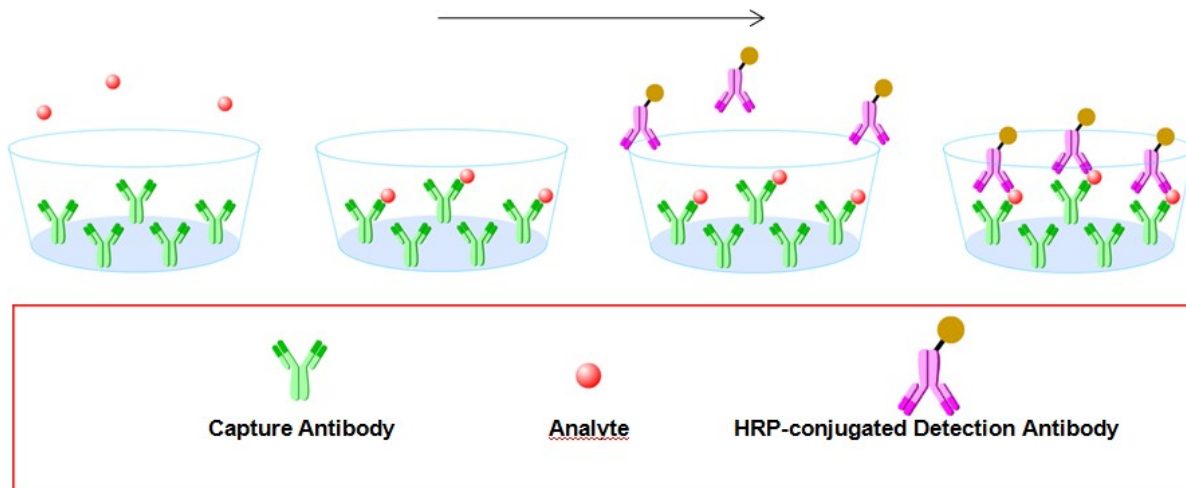
PRINCIPLE OF THE TEST

The Novus Biologicals Serpin A1/alpha 1-Antitrypsin Antibody Pair [HRP] is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for Serpin A1/alpha 1-Antitrypsin coated on a 96-well plate. Standards and samples are added to the wells, and any Serpin A1/alpha 1-Antitrypsin present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated rabbit anti- Serpin A1/alpha 1-Antitrypsin monoclonal 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 A1/alpha 1-Antitrypsin 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 Human Serpin A1/alpha 1-Antitrypsin Antibody Pair [HRP] is for the quantitative determination of Human Serpin A1/alpha 1-Antitrypsin.
- ◆ This Serpin A1/alpha 1-Antitrypsin 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 – 0.5 mg/mL of mouse anti-Serpin A1/alpha 1-Antitrypsin monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 1 µg/mL in PBS before coating.

Detection Antibody – 0.2 mg/mL rabbit anti-Serpin A1/alpha 1-Antitrypsin monoclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4). Dilute to working concentration of 0.4 µg/mL in detection antibody dilution buffer before use.

Standard – Each vial contains 160 ng of recombinant Serpin A1/alpha 1-Antitrypsin. Reconstitute 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 2 ng/mL is recommended.

SOLUTIONS REQUIRED

PBS - 136.9 mM NaCl, 10.1 mM Na₂HPO₄, 2.7 mM KCl, 1.8 mM KH₂PO₄, pH 7.4, 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: Store at 4°C and protect it from prolonged exposure to light for up to 6 months from date of receipt. **DO NOT FREEZE!**

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 PBS. 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

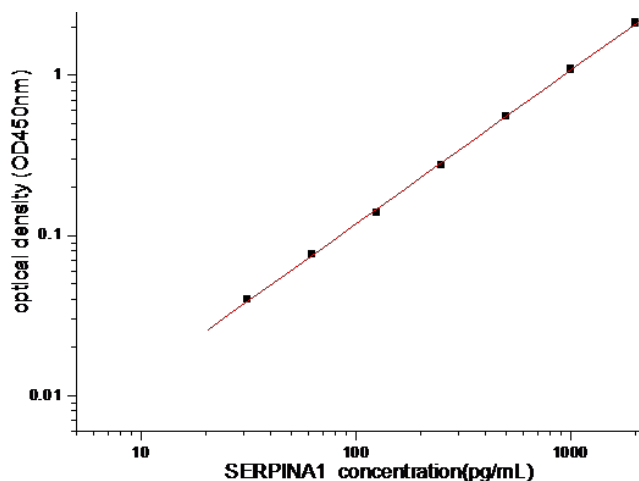
1. Add 100 µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate 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
31.25	0.040
62.5	0.076
125	0.138
250	0.275
500	0.552
1000	1.091
2000	2.118

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of Human Serpin A1/alpha 1-Antitrypsin was determined to be approximately 31.25 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
	The concentration of samples was too high	Try higher dilution rate of samples

ELISA Plate Template

[illegible]

**Human Serpin A1/alpha 1-Antitrypsin
Antibody Pair [HRP]**

Notes