ab108799 Human alpha 1 Antitrypsin (SERPINA1) ELISA Kit

For the quantitative measurement of human alpha 1 Antitrypsin in plasma, serum, milk, urine, saliva and CSF samples.

This product is for research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab108799

Materials Supplied:

Item	Quantity	Storage Condition
alpha 1 Antitrypsin Microplate (12 x 8 wells)	96 wells	4°C
alpha 1 Antitrypsin Standard	1 vial	4°C
10X Diluent N Concentrate	30 mL	4°C
Biotinylated human alpha 1 Antitrypsin Antibody	1 vial	-20°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	7 mL	4°C
Stop Solution	11 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Sealing Tapes	3	N/A

Storage and Stability:

Store kit at +4°C immediately upon receipt, apart from the SP Conjugate & Biotinylated Antibody, which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Required, Not Supplied:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 6 tubes to prepare standard or sample dilutions.

Reagent Preparation:

When diluting the concentrates, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have dissolved.

1X Diluent N: Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. Store for up to 1 month at 4°C.

1X Wash Buffer: Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

1X Biotinylated alpha 1 Antitrypsin Detector Antibody:

1) The stock Biotinylated alpha 1 Antitrypsin Antibody must be diluted with 1X Diluent N according to the label concentration to prepare 1X Biotinylated alpha 1 Antitrypsin Antibody for use in the assay procedure. Observe the label for the "X" concentration on the vial of Biotinylated alpha 1 Antitrypsin Antibody.
2) Calculate the necessary amount of 1X Diluent N to dilute the Biotinylated alpha 1 Antitrypsin Antibody to prepare a 1X Biotinylated alpha 1 Antitrypsin Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation:

Number of Wells Strips	Number of Wells	(V_T) Total Volume of 1X Biotinylated Detector Antibody (µL)
4	32	1,760
6	48	2,640
8	64	3,520
10	80	4,400
12	96	5,280

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells: $\mathbf{(C_F / C_S) \times V_T = V_A}$

Calculate the final volume of 1X Diluent N required to prepare the 1X Biotinylated alpha 1 Antitrypsin Detector Antibody: $\mathbf{V_T - V_A = V_D}$

Where:

C_S = Starting concentration (X) of stock Biotinylated alpha 1 Antitrypsin Antibody (variable)

C_F = Final concentration (always = 1X) of 1X Biotinylated alpha 1 Antitrypsin Detector Antibody solution for the assay procedure

V_T = Total required volume of 1X Biotinylated alpha 1 Antitrypsin Detector Antibody solution for the assay procedure

V_A = Total volume of (X) stock Biotinylated alpha 1 Antitrypsin Antibody

V_D = Total volume of 1X Diluent N required to dilute (X) stock Biotinylated alpha 1 Antitrypsin Antibody to prepare 1X Biotinylated Detector Antibody solution for assay procedures

3) Spin the Biotinylated alpha 1 Antitrypsin Antibody vial to collect contents at the bottom.
4) Add calculated amount V_A of stock Biotinylated alpha 1 Antitrypsin Antibody to the calculated amount V_D of 1X Assay Diluent N. Mix gently and thoroughly.

1X SP Conjugate Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N. Any remaining solution should be frozen at -20°C.

Standard Preparation:

Always prepare a fresh set of standards for every use. Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.

1) Reconstitute the alpha 1 Antitrypsin Standard vial to generate a 25 ng/mL alpha 1 Antitrypsin **Standard #1**.
   1.1) First consult the alpha 1 Antitrypsin Standard vial to determine the mass of protein in the vial.
   1.2) Calculate the appropriate volume of 1X Diluent N to add when resuspending the alpha 1 Antitrypsin Standard vial to produce a 25 ng/mL alpha 1 Antitrypsin **Standard #1** by using the following equation:
   C_S = Starting mass of alpha 1 Antitrypsin Standard (see vial label) (ng)
   C_F = 25 ng/mL alpha 1 Antitrypsin **Standard #1** final required concentration
   V_D = Required volume of 1X Diluent N for reconstitution (µL)
   Calculate total required volume 1X Diluent N for resuspension: $\mathbf{(C_S / C_F) \times 1{,}000 = V_D}$
   1.3) First briefly centrifuge the alpha 1 Antitrypsin Standard Vial to collect the contents on the bottom of the tube.
   1.4) Reconstitute the alpha 1 Antitrypsin Standard vial by adding the appropriate calculated amount V_D of 1X Diluent N to the vial to generate the 25 ng/mL alpha 1 Antitrypsin **Standard #1.** Mix gently and thoroughly.
2) Allow the reconstituted 25 ng/mL alpha 1 Antitrypsin **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
3) Label five tubes #2 – 8.
4) Add 120 µL of 1X Diluent N to tube #2 – 8.

5) To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.
6) To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.
7) Using the table below as a guide, prepare subsequent serial dilutions.
8) 1X Diluent N serves as the zero standard (0 ng/mL) (tube #8).

Standard #	Volume to Dilute (µL)	Volume Diluent N (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Step 2.1				25
2	120	120	240	25	12.5
3	120	120	240	12.5	6.25
4	120	120	240	6.25	3.125
5	120	120	240	3.125	1.563
6	120	120	240	1.563	0.781
7	120	120	240	0.781	0.391
8	-	120	120		0

Sample Preparation
Avoid repeated freeze-thaw cycles for all.

Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. A 200000-fold sample dilution is suggested into 1X Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 200000-fold sample dilution is suggested into 1X Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. A 2000-fold sample dilution is suggested into 1X Diluent N or within the range of 100x – 8000x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. A 20-fold sample dilution is suggested into 1X Diluent N or within the range of 2x – 200x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. A 400-fold sample dilution is suggested into 1X Diluent N or within the range of 40x – 4000x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. A 4000-fold sample dilution is suggested into 1X Diluent N or within the range of 40x – 40000x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Assay Procedure:
Equilibrate all materials and prepared reagents to RT prior to use. Assay all in duplicate
1) Prepare all reagents, working standards, and samples as directed in the previous sections. The assay is performed at room temperature (18-25°C).
2) Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3) Add 50 µL of alpha 1 Antitrypsin Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.
4) Wash five times with 200 µL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to remove the liquid.
5) Add 50 µL of 1X Biotinylated alpha 1 Antitrypsin Antibody to each well and incubate for 1 hour.
6) Wash the microplate as described above.
7) Add 50 µL of 1X Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8) Wash the microplate as described above.
9) Add 50 µL of Chromogen Substrate per well and incubate in ambient light for about 30 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
10) Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.
11) Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

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