HUMAN ALPHA-2 ANTIPLASMIN (A2AP)/ SERPIN F2 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ALPHA-2 ANTIPLASMIN /SERPIN F2 CONCENTRATIONS IN SERUM AND PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECL ALL ITEM OF EACH KIT BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN ALPHA-2 ANTIPLASMIN / SERPIN F2 ELISA KIT**
Catalog No.	SK00256-01
Lot No.:	
Formulation	96 T
Standard range	0.25 – 16 ng/mL
Sensitivity	30 pg/mL
Sample Volume	100 µL
Dilution Factor	8000 or higher (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum and EDTA Plasma
Specificity	Human
Calibration	Human Serpin F2 recombinant (HEK293)
Intra-assay Precision	3-6%
Inter-assay Precision	4-8%
Storage	2 – 8° C for 10 month
This kit contains sufficient materials to run approximately 40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Alpha-2 Antiplasmin (A2AP)/Serpin F2 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Alpha-2 Antiplasmin from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Alpha-2 Antiplasmin derived from HEK293 and Monoclonal antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Alpha-2 Antiplasmin samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human Alpha-2 Antiplasmin. The capture antibody can bind to the human Alpha-2 Antiplasmin in the standard and samples. After washing the plate of any unbound substances, a monoclonal antibody-HRP conjugate against human Alpha-2 Antiplasmin is added to the wells. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human Alpha-2 Antiplasmin bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Alpha-2 Antiplasmin Microplate – 96 well microplate precoated with an antibody specific for human Alpha-2 Antiplasmin.	**256-01-01**	**1 plate**
Alpha-2 Antiplasmin Standard – 8000 pg/vial of lyophilized recombinant human Alpha-2 Antiplasmin.	**256-01-02**	**2 vials**
Detection Antibody-HRP Conjugate – 55 µL/vial of 200-fold concentrated solution of antibody conjugated to HRP against Alpha-2 Antiplasmin.	**256-01-03**	**1 vial**
Positive Control – one vial of lyophilized recombinant human Alpha-2 Antiplasmin.	**256-01-04**	**1 vial**
Dilution Buffer - 45 mL of buffered solution with preservative.	**DB10**	**3 bottles**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 10 months. For longer storage, unopened Standard and Positive Control should be stored at -20° C or -70° C.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (350 – 400 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum or plasma require the final 8000 (8K) or higher dilution. Please refer page 5 of 2000-fold pre-diluted sample solutions.

Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.

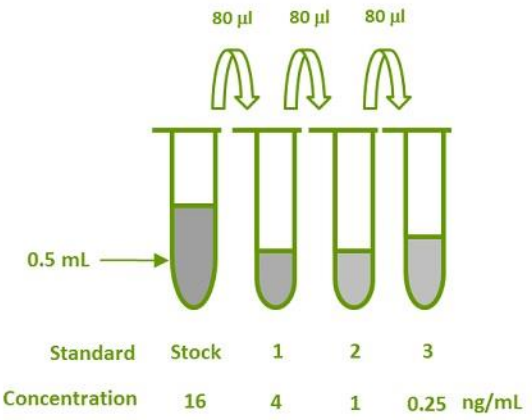
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Alpha-2 Antiplasmin Standard - Reconstitute the Alpha-2 Antiplasmin standard with **0.5 mL** of Dilution Buffer. This reconstitution produces a stock solution of 16 ng/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next 4-fold transfer. The **16 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	0.5 ml	16 ng/ml
# 1	80 µl of stock	240 µl	4 ng/ml
# 2	80 µl of 1	240 µl	1 ng/ml
# 3	80 µl of 2	240 µl	0.25 ng/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer.

Detection Antibody-HRP Conjugate - Pipette 10.945 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 55 µL of 200-fold concentrated stock solution to prepare working solution **(protect from light)**. **DO NOT FREEZE.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µl per well of Dilution Buffer to Blank wells. **Add 75 µl per well of Dilution Buffer (DB10) to all samples wells by Multi-channel Pipette .**
3. Add 100 µl per well of standard dilutions from #3 to #S (reverse order of serial dilution),

positive control. **Add 25 µl per well of 2000-fold diluted samples to all sample wells. (The final dilution factor for sample is 8000-fold (8K)**. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (400 rpm).

4. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
5. Add 100 µl per well of 1x Detection Antibody-HRP conjugate working solution. Cover with plate sealer and incubate at room temperature for 1 hour on microplate shaker (400 rpm). **Protect from light.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Substrate Solution to each well. Incubate for 22-28 minutes on microplate shaker at room temperature. **Protect from light**.
8. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well using a microplate reader set to 450 nm within 3 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log or 4-parameter curve fit to more accurately quantify the standard dilutions.

If samples have been diluted by 8000, the concentration read from the standard curve must be multiplied by the dilution factor 8000.

TYPICAL DATA

This standard curve data is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450 NM (CORRECTED)
Blank	0 (0.060)
0.25	0.055
1	0.219
4	0.803
16	2.299

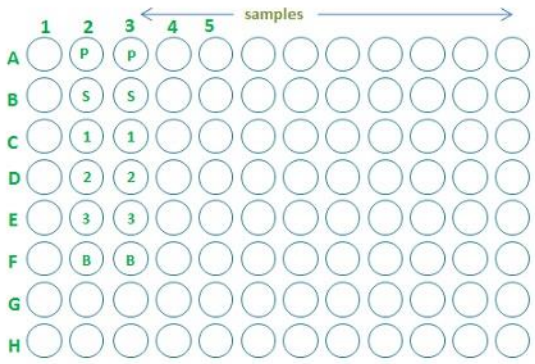
SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human Alpha-2 Antiplasmin (HEK293)	100%
Human Vaspin	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, or positive control to the well. Add 75µl of Dilution Buffer into all samples wells and add 25 µl of 2000-fold pre-diluted sample solution into all samples wells. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl per well 1x Detection Antibody-HRP working solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl TMB Substrate Solution to each well. Incubate 22-28 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 3 min.

*Due the concentration of Serpin F2 in human serum or plasma samples is **very high and out of standard curve**, all serum or plasma samples require 8000-fold or higher dilution prior to perform assay.*



Optimal dilutions should be determined by each laboratory for each application with a pretest.
Use polypropylene test tubes.

How to pre-dilute serum or plasma samples use 10 µL:

Serum and plasma samples may require a 8000-fold or higher dilution. A suggested 50-fold dilution is **10 µL sample** +**490 µL** of Dilution Buffer. Then, to make a final 2000-fold dilution is **10 µL of 50-fold diluted** sample + **390 µL** of Dilution Buffer.
Final 8000-fold dilution is **adding 25 µL of 2000-fold pre-diluted sample solution plus 75 µL of Dilution Buffer DB10 on all sample wells.** The final dilution factor is 8000.

Dilution Factor	Sample	Dilution Buffer (DB10)
50-fold	10 µL	490 µL
2000-fold	10 µL of 50-fold diluted sample solution	390 µL
8000-fold	**Add 25 µL of 2000-fold pre-diluted samples to all samples wells**	**Add 75 µl per well of Dilution Buffer to all samples wells by Multi-channel Pipette**
(optional 16000-fold)	Add 12.5 µL of 2000-fold pre-diluted samples to all samples wells	Add 87.5 µl per well of Dilution Buffer to all samples wells by Multi-channel Pipette