

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

FOR THE QUANTITATIVE DETERMINATION  
OF MOUSE ALPHA-2 ANTIPLASMIN  
CONCENTRATIONS IN SERUM AND  
PLASMA



ALWAYS REFER TO LOT SPECIFIC PROTOCOL  
PROVIDED WITH EACH KIT FOR  
INSTRUCTIONS. PROTOCOL MUST BE  
READ BEFORE USING THIS PRODUCT.

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

### PRODUCT INFORMATION:

ELISA NAME	MOUSE ALPHA-2 ANTIPLASMIN ELISA
Catalog No.	SK00256-03
Lot No.:	
Formulation	96 T
Standard range	46-3000 pg/mL
Sensitivity	20 pg/mL
Sample Volume	100 µL
Dilution Factor	<b>20000 (Optimal dilutions should be determined by each laboratory for each application)</b>
Sample Type	Serum and EDTA Plasma
Specificity	Mouse Alpha-2 Antiplasmin
Calibration	Mouse Alpha-2 Antiplasmin recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 - 8° C
<b>This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.</b>	

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## DESCRIPTION

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

This immunoassay contains recombinant mouse Alpha-2 Antiplasmin and 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 an antibody specific for mouse Alpha-2 Antiplasmin. The capture antibody can bind to the mouse Alpha-2 Antiplasmin in the standard and samples. After washing the plate of any unbound substances, an antibody-HRP conjugate against mouse 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 mouse 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 with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

\_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
<b>Alpha-2 Antiplasmin Microplate</b> – 96 well microplate precoated with an antibody specific for mouse Alpha-2 Antiplasmin.	<b>256-03-01</b>	<b>1 plate</b>
<b>Alpha-2 Antiplasmin Standard</b> – refer to package label vial of lyophilized recombinant mouse Angiotensinogen.	<b>256-03-02</b>	<b>1 vial</b>
<b>Detection Antibody-HRP Conjugate</b> – refer to package label of 100-fold concentrated solution of antibody conjugated to HRP against Angiotensinogen.	<b>256-03-03</b>	<b>1 vial</b>
<b>Positive Control</b> – one vial of lyophilized recombinant mouse Angiotensinogen.	<b>256-03-04</b>	<b>1 vial</b>
<b>Dilution Buffer</b> - 60 mL of buffered solution with preservative.	<b>DB10</b>	<b>2 bottles</b>
<b>Wash Buffer</b> - 50 mL of 10-fold concentrated buffered surfactant with preservative.	<b>WB01</b>	<b>1 bottle</b>
<b>TMB Substrate Solution</b> - 11 mL of TMB substrate solution.	<b>TMB01</b>	<b>1 bottle</b>
<b>Stop Solution</b> - 11 mL of 0.5M HCl.	<b>S-STOP</b>	<b>1 bottle</b>
<b>Plate Sealer</b>	<b>EAPS</b>	<b>1 piece</b>
<b>Plastic Pouch</b>	<b>P01</b>	<b>1 piece</b>

## STORAGE

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

Substrate Solution can be stored at 2 – 8° C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components can be stored at 2 – 8° C for up to 8 months. Do not use kit past expiration date.

**Opened / Reconstituted Reagents:** Reconstituted Standard (stock) solution SHOULD BE STORED at -20° C or -70° C for up to one month.

**Microplate Wells:** Return unused microplate strips to the plastic pouch with the desiccant pack.

Microplate may be stored for up to 6 months at 2 – 8° C after opening.

**ADDITIONAL MATERIALS REQUIRED**

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 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  $\leq -20^{\circ}\text{C}$ . 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  $\leq -20^{\circ}\text{C}$ . 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 and plasma samples may need a 20000-fold dilution. A suggested 50-fold dilution is 10  $\mu\text{L}$  sample + 490  $\mu\text{L}$  Dilution Buffer. Then, to make a 2500-fold dilution is 10  $\mu\text{L}$  of 50-fold diluted sample + 490  $\mu\text{L}$  Dilution Buffer. Finally, to make a 20000-fold dilution is 40  $\mu\text{L}$  of 2500-fold diluted sample + 280  $\mu\text{L}$  Dilution Buffer.

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 Angiotensinogen standard with refer to package label of Dilution Buffer. This reconstitution produces a stock solution of 3000 pg/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 transfer. The **3,000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	Refer to package label	3000 pg/ml
# 1	250 $\mu\text{L}$ of stock	250 $\mu\text{L}$	1500 pg/ml
# 2	250 $\mu\text{L}$ of 1	250 $\mu\text{L}$	750 pg/ml
# 3	250 $\mu\text{L}$ of 2	250 $\mu\text{L}$	375 pg/ml
# 4	250 $\mu\text{L}$ of 3	250 $\mu\text{L}$	187.5 pg/ml
# 5	250 $\mu\text{L}$ of 4	250 $\mu\text{L}$	93.5 pg/ml
# 6	250 $\mu\text{L}$ of 5	250 $\mu\text{L}$	46.88 pg/ml

**Positive Control** - Reconstitute the Positive Control with refer to package label mL of Dilution Buffer.

**Note:** Positive Control could be reused within a few days if stored at  $-20^{\circ}\text{C}$  or  $-70^{\circ}\text{C}$ .

**Detection Antibody-HRP Conjugate** - Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105  $\mu\text{L}$  of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Detection Antibody-HRP conjugate should be used within a few days (**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. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µl per well of Dilution Buffer to Blank wells.
4. Add 100 µl per well of standard dilutions from #6 to #S (reverse order of serial dilution), positive control or samples. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
5. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. 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 (250 rpm).  
**Protect from light.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 5-10 minutes on microplate shaker at room temperature. **Protect from light.**
9. 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.
12. Determine the optical density of each well within 15 minutes using a microplate reader set to 450 nm.

## 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 curve fit to more accurately quantify the standard dilutions.

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

## 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 (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.108)
46.88	0.041
93.75	0.089
187.5	0.138
375	0.274
750	0.508
1500	0.845
3000	1.399

## SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Mouse Alpha-2 Antiplasmin	100%
Mouse Vaspin	0

## SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
 Add 100 µl of standard dilutions, samples, or positive control to the well. 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. <b>Protect from light.</b>
 Aspirate and wash 4 times.
 Add 100 µl TMB Substrate Solution to each well. Incubate 5-10 min on the plate shaker at RT. <b>Protect from light.</b>
 Add 100 µl Stop Solution to each well. Read 450nm within 15 min.