Active Mouse α-2-Antiplasmin ELISA

For Research Use Only

INTRODUCTION

This mouse α-2-antiplasmin ELISA is for the quantitative determination of active α-2-antiplasmin in mouse plasma.

α-2-antiplasmin (α2AP) is the major circulating inhibitor of plasmin, and it has a role in the regulation of intravascular fibrinolysis[1,2]. Decreased levels of α-2-antiplasmin may play an important role in the increased capacity of the fibrinolytic function and may be beneficial in the treatment of thrombotic diseases, acute pulmonary embolism, and hepatic repair[3,4,6,7].

PRINCIPLES OF PROCEDURE

Functionally active α-2-antiplasmin present in plasma reacts with plasmin that has been coated and dried on a microtiter plate. Latent or complexed α-2-antiplasmin will not bind to the plate or be detected. Unbound α-2-antiplasmin is removed by washing and an anti-α-2-antiplasmin primary antibody is added. Excess primary antibody is removed by washing. The bound antibody, which is proportional to the original active α-2-antiplasmin present in the samples, is then reacted with the horseradish peroxidase conjugated streptavidin. Following an additional washing step, TMB substrate solution is then used for color development at 450 nm. The amount of color development is directly proportional to the concentration of active α-2-antiplasmin in the sample.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	Plasmin coated 96-well plate	1 plate	4°C	PL98a
Standard	Mouse α-2-antiplasmin activity standard (lyophilized)	1 vial	4°C	PL98b
Wash Buffer	10x solution for washing plate	50 mL	4°C	PL98c
Primary Antibody	Anti-mouse α-2-antiplasmin antibody (lyophilized)	1 vial	4°C	PL98d
Streptavidin-HRP	HRP conjugated streptavidin	1 vial	4°C	PL98e
Substrate	TMB Substrate	10 mL	4°C	PL98f

MATERIALS NEEDED BUT NOT PROVIDED

1. Pipettes covering 0-10 μl and 200-1000 μl and tips
2. 12-channel pipette covering 30-300 μl
3. 1 N H_2SO_4
4. DI water
5. Microtiter plate spectrophotometer with a 450 nm filter
6. Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

STORAGE CONDITIONS

1. Store this kit and its components at 4°C until use.

PROCEDURAL NOTES

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions, or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.
3. Exercise universal precautions during the performance or handling of this kit or any component contained therein.

SAMPLE COLLECTION AND PREPARATION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid repeated freeze-thaw cycles. It is highly suggested to dilute unknowns 1:10,000 – 1:20,000 in 3% BSA Blocking Buffer because of the high level of α-2-antiplasmin in normal mouse plasma and serum. This assay measures active α-2-antiplasmin in the 0.1 - 100 ng/ml range.

REAGENT PREPARATION

1. **10x Wash Buffer:** Dilute the 50 mL of concentrate to 1x with 450 mL of DI water prior to use.
2. **TBS Buffer:** 0.1 M Tris, 0.15 M NaCl, pH 7.4.
3. **3% BSA Blocking Buffer:** 3% (w/v) BSA in TBS Buffer.
4. **Standard:** Reconstitute with 10 mL of 3% BSA Blocking Buffer directly to the vial for a 1000 ng/mL solution and vortex gently to mix. Prepare immediately prior to use and proceed to the table below.
5. **Primary Antibody:** Reconstitute with 10.0 mL 3% BSA Blocking Buffer and vortex gently to mix. Prepare immediately prior to use.
6. **Streptavidin-HRP:** Dilute 2.5uL of Streptavidin-HRP into 2.5 mL 3% BSA Blocking Buffer and vortex gently to mix to make a 1:1,000 dilution. Add 0.4 mL of the 1:1000 dilution to 9.6 mL 3% BSA Blocking Buffer to make a 1:25,000 dilution. Prepare immediately prior to use.

SANDARD PREPARATION

Table 1: Preparation of Standard Curve

Standard	α2AP Concentration (ng/mL)	Blocking Buffer (µL)	Transfer Volume (µL)	Transfer Source	Final Volume (µL)
S_{10}	100	900	100	Stock Vial	500
S_9	50	500	500	S_{10}	500
S_8	25	500	500	S_9	600
S_7	10	600	400	S_8	500
S_6	5	500	500	S_7	500
S_5	2.5	500	500	S_6	600
S_4	1.0	600	400	S_5	500
S_3	0.5	500	500	S_4	500
S_2	0.25	500	500	S_3	600
S_1	0.1	600	400	S_2	1,000
B_0	0	500	---	---	500

ASSAY PROCEDURE

1. Add 100 μl of the Standards and unknowns to the wells in duplicate. Shake the plate at 300 rpm for 30 minutes at room temperature (RT). For a suggested plate layout, see Scheme I below.
2. Wash the plate 3 times according to the following wash procedure:
   a. Remove the contents of each well by inversion of the plate.
   b. Tap out the remaining contents of the plate onto a lint free paper towel.
   c. Add 300 μL of 1x Wash Buffer to each well.
   d. Let stand for 2 minutes.
   e. Repeat procedure two more times, then proceed to step "f".
   f. Remove the contents of each well by inversion of plate into an appropriate disposal device.
   g. Tap out the remaining contents of the plate onto a lint free paper towel, then proceed to step 3.
3. Add 100 μl of the Primary Antibody to each well. Shake the plate at 300 rpm for 30 minutes at RT.
4. Wash the plate three times as in step 2.
5. Add 100 μl of the Streptavidin-HRP to each well. Shake the plate at 300rpm for 30 minutes at RT.
6. Wash the plate three times as in step 2.
7. Add 100 μl of TMB Substrate to each well. Shake the plate at 300 rpm for 2-10 minutes at RT.
8. Stop the reaction by adding 50 μl of 1N H_2SO_4 to each well and read the plate at 450 nm.

Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_{10}	S_9	S_8	S_7	S_6	S_5	S_4	S_3	S_2	S_1	B_0	U_2
B	S_{10}	S_9	S_8	S_7	S_6	S_5	S_4	S_3	S_2	S_1	B_0	U_2
C	U_2	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}
D	U_2	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}
E	U_{14}	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}
F	U_{14}	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}
G	U_{26}	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}
H	U_{26}	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}

CALCULATIONS

1. Plot the A_{450} against the concentration of α-2-antiplasmin in the standards.
2. Fit a straight line through the points using a linear fit procedure.
3. Calculate the α-2-antiplasmin concentrations in the unknowns using the equation generated by the standard curve. If the samples were diluted, be sure to multiply by the dilution factor.

EXPECTED VALUES

It has been determined in laboratory testing that mouse plasma contains approximately 86 μg/ml α-2-antiplasmin.

Abnormalities in α-2-antiplasmin levels have been reported in the following conditions:

- Hemostatic Dysfunction: Low levels of α-2-antiplasmin may result in hemostatic dysfunction[5].
- Thrombus Formation: Reduction of α-2-antiplasmin may result in thrombus formation[8].

REFERENCES

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