Catalog # MA2APKT

Mouse Alpha-2-antiplasmin activity assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This mouse alpha-2-antiplasmin activity assay is for the quantitative determination of active alpha-2-antiplasmin in mouse plasma.

BACKGROUND

Alpha2-antiplasmin (a2AP) is the major circulating inhibitor of plasmin. Its role is in the regulation of intravascular fibrinolysis [1,2]. Decreased levels of alpha2-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].

ASSAY PRINCIPLE

Functionally active alpha2-antiplasmin present in plasma reacts with plasmin coated and dried on a microtiter plate. Latent or complexed alpha2-antiplasmin will not bind to the plate or be detected. Unbound alpha2-antiplasmin samples are aspirated and an anti-alpha2-antiplasmin primary antibody is added. Excess primary antibody is then aspirated. The bound antibody, which is proportional to the original active alpha2-antiplasmin present in the samples, is then reacted with the horseradish peroxidase conjugated secondary antibody. Following an additional washing step, TMB substrate solution is then used for color development at 450nm. The amount of color development is directly proportional to the concentration of active alpha2-antiplasmin in the sample.

REAGENTS PROVIDED

♦Immunoassay plate:
1-96 well immulon plate coated, blocked, and dried with plasmin
♦Mouse alpha2-antiplasmin activity standard:
1 vial of lyophilized standard
♦10X Wash Buffer:
1 bottle of 50ml wash; bring to 1X using DI water
♦Anti-mouse alpha2-antiplasmin primary antibody:
1 vial lyophilized polyclonal anti-mouse alpha2-antiplasmin antibody
♦Horseradish peroxidase secondary antibody: 1 vial concentrated HRP labeled antibody
♦TMB One substrate solution:
1 bottle 10 ml solution

STORAGE AND STABILITY

Kit components should be stored at 4°C when not in use. Kit should be used no later then the expiration date.

REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette for 30-300µl
- Paper towels or kimwipes
- 50ml tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer
- Blocking buffer
- Microtiter plate spectrophotometer operable at 450nm

•Microtiter plate shaker with uniform horizontally circular movement up to 300rpm

WARNINGS

Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

PRECAUTIONS

•**DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
•**DO NOT** pipette reagents by mouth.
•Always pour substrate out of the bottle into a clean test tube. DO NOT pipette out of the bottle as you could contaminate the substrate.
•Keep plate covered except when adding reagents, washing, or reading.
•All kit components must be kept refrigerated (4°C).
•**DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

•**TBS buffer:** 0.10M TRIS, 0.15M NaCl, pH 7.4
•**Blocking buffer:** 3% BSA in TBS buffer

SPECIMEN COLLECTION

This assay has been validated for use with samples of mouse plasma in citrate anticoagulant and mouse serum. Collect 9 volumes of blood in 1 volume of 0.1M trisodium citrate or acidified citrate. Immediately after collection of blood, samples must be centrifuged at 3000Xg for 15 minutes. The plasma should be transferred to a clean plastic tube and must be stored on ice prior to analysis. The samples are stable on ice for up to 6 hours or freeze at –20°C or colder for extended storage.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:

Reconstitute standard as directed on vial and agitate gently to completely dissolve contents.
Mouse alpha-2-antiplasmin: 10µg/ml before reconstitution

MA2AP-FT concentration (µg/ml)	Dilutions
10	100µl straight (from vial)
5	500µl (BSA) + 500µl (10µg/ml)
2.5	500µl (BSA) + 500µl (5µg/ml)
1	600µl (BSA) + 400µl (2.5µg/ml)
0.5	500µl (BSA) + 500µl (1µg/ml)
0.25	500µl (BSA) + 500µl (0.5µg/ml)
0.1	600µl (BSA) + 400µl (0.25µg/ml)
0.05	500µl (BSA) + 500µl (0.1µg/ml)
0.025	500µl (BSA) + 500µl (0.05µg/ml)

NOTE: DILUTIONS FOR THE STANDARD CURVE MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:

Add 100µl standard in duplicate and unknown to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.
NOTE: If the unknown is thought to have high mouse alpha2-antiplasmin levels, dilutions may be made in 3% BSA blocking buffer.

Primary Antibody Addition:

Reconstitute primary antibody as directed on vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for

30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

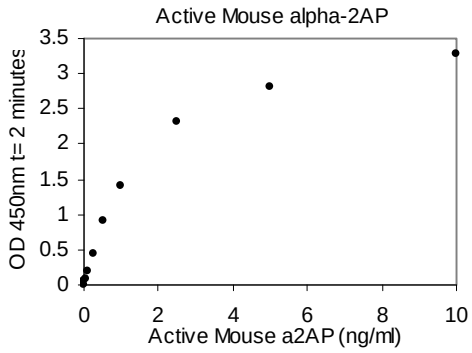
Secondary Antibody Addition:
Dilute 2.5µl into 10ml 3% BSA and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

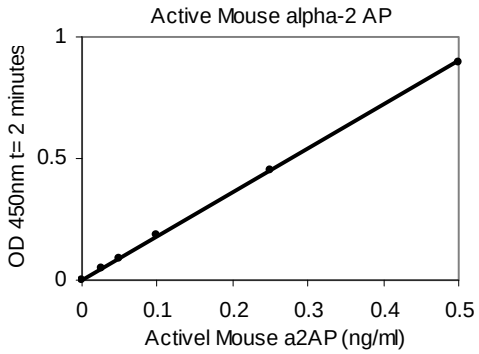
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Quench the reaction with the addition of 50µl of 1N H_2SO_4 and read final absorbance values at 450nm.
NOTE: Time for substrate development is dependent on needs of researcher.

Measurement:
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm, A_{450}.

Assay Calibration:
Plot A_{450} against the amount of alpha2-antiplasmin in the standards. Fit a straight line through the points using a linear fit procedure. The alpha2-antiplasmin activity in the unknowns can be determined from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





EXPECTED VALUES

It has been determined in laboratory testing that mouse plasma contains approximately 300µg/ml alpha2-antiplasmin.

Abnormalities in alpha2-antiplasmin levels have been reported in the following condition:

♦ Hemostatic Dysfunction: Low levels of alpha2-antiplasmin may result in hemostatic dysfunction [5].

♦ Thrombus Formation: Reduction of alpha2-antiplasmin may result in thrombus formation [8].

PERFORMANCE CHARACTERISTICS

The assay measures active alpha2-antiplasmin in the 0-10 µg/ml range. Extensive dilutions must be performed to assure the unknowns will be in the assay range. It is highly suggested to dilute unknowns at 1:1000 because of the high level of alpha2-antiplasmin in normal murine plasma and serum.

DISCLAIMER

This information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

REFERENCES

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