Mouse Alpha-2-Antiplasmin Total ELISA Kit

Catalog No: IMA2APKT-TOT
Lot No: 413

INTENDED USE
This mouse α2-antiplasmin assay is for the quantitative determination of total α2-antiplasmin in mouse plasma. For research use only.
BACKGROUND
α2-antiplasmin is the major circulating inhibitor of plasmin. It plays a role in the regulation of intravascular fibrinolysis [1,2]. Decreased levels of α2-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
Total mouse α2-antiplasmin will bind to the affinity purified capture antibody coated on the microtiter plate. Complexed and free α2-antiplasmin will react with the antibody on the plate. After appropriate washing steps, polyclonal anti-mouse α2-antiplasmin primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody, which is proportional to the total α2-antiplasmin present in the samples, is reacted with horseradish peroxidase conjugated streptavidin. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of mouse α2-antiplasmin. Color development is proportional to the concentration of α2-antiplasmin in the samples.

REAGENTS PROVIDED
- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-mouse α2-antiplasmin antibody, blocked and dried.
- **10X Wash Buffer**: 1 bottle of 50ml
- **Mouse α2-antiplasmin standard**: 1 vial lyophilized standard
- **Anti-mouse α2-antiplasmin primary antibody**: 1 vial lyophilized polyclonal antibody
- **Horseradish peroxidase-conjugated Streptavidin**: 1 vial concentrated HRP labeled streptavidin
- **TMB substrate solution**: 1 bottle of 10ml solution

STORAGE AND STABILITY
Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED
- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H₂SO₄ or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)
PRECAUTIONS
• FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
• 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.
• Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
• Keep plate covered except when adding reagents, washing, or reading.
• DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
• DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS
• TBS buffer: 0.1M Tris, 0.15M NaCl, pH 7.4
• Blocking buffer (BB): 3% BSA (w/v) in TBS
• 1X Wash buffer: Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water.

SAMPLE COLLECTION
Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

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

Preparation of Standard
Reconstitute standard by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1000ng/ml standard solution.

Dilution table for preparation of mouse α2-antiplasmin standard:

<table>
<thead>
<tr>
<th>α2-antiplasmin concentration (ng/ml)</th>
<th>Dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>900μl (BB) + 100μl (from vial)</td>
</tr>
<tr>
<td>50</td>
<td>500μl (BB) + 500μl (100ng/ml)</td>
</tr>
<tr>
<td>25</td>
<td>500μl (BB) + 500μl (50ng/ml)</td>
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<tr>
<td>10</td>
<td>600μl (BB) + 400μl (25ng/ml)</td>
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<td>500μl (BB) + 500μl (10ng/ml)</td>
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<td>1</td>
<td>600μl (BB) + 400μl (2.5ng/ml)</td>
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<td>500μl (BB) + 500μl (1ng/ml)</td>
</tr>
<tr>
<td>0.25</td>
<td>500μl (BB) + 500μl (0.5ng/ml)</td>
</tr>
<tr>
<td>0.1</td>
<td>600μl (BB) + 400μl (0.25ng/ml)</td>
</tr>
<tr>
<td>0</td>
<td>500μl (BB) Zero point to determine background</td>
</tr>
</tbody>
</table>

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.
Standard and Unknown Addition
Remove microtiter plate from bag and add 100µl α2-antiplasmin standards (in duplicate) and unknowns 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: The assay measures α2-antiplasmin antigen in the 0.1-100 ng/ml range. If the unknown is thought to have high α2-antiplasmin levels, dilutions may be made in blocking buffer. A 1:10,000-1:20,000 dilution for normal mouse plasma is suggested for best results.

Primary Antibody Addition
Reconstitute primary antibody by adding 10ml of blocking buffer directly to the 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.

Streptavidin-HRP Addition
Dilute 2.5µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.2ml of 1:1,000 dilution to 9.8ml of blocking buffer to generate a 1:50,000 dilution. Add 100µl of the 1:50,000 dilution 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 2-5 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H2SO4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

Measurement
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A450).
Calculation of Results
Plot $A_{450}$ against the amount of $\alpha_2$-antiplasmin in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of $\alpha_2$-antiplasmin in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):

EXPECTED VALUES
The normal human concentration of $\alpha_2$-antiplasmin is 70µg/ml or 1µM in plasma and 47.6 µg/ml or 0.68µM in serum [8]. The normal mouse concentration of $\alpha_2$-antiplasmin is 86µg/ml in plasma [9]. In house testing for mouse $\alpha_2$-antiplasmin levels in normal pooled plasma found 80µg/ml.

Abnormalities in $\alpha_2$-antiplasmin levels have been reported in the following conditions:
• Hemostatic Dysfunction: Low levels of $\alpha_2$-antiplasmin may result in hemostatic dysfunction [5].
• Thrombus Formation: Reduction of $\alpha_2$-antiplasmin may result in thrombus formation [10].
PERFORMANCE CHARACTERISTICS

Sensitivity: These studies are currently in progress. Please contact us for more information.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: These studies are currently in progress. Please contact us for more information.

DISCLAIMER

This information is believed to be correct but does not claim 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 of or contact with the above product.

REFERENCES

### Example of ELISA Plate Layout

**96 Well Plate: 22 Standard wells, 74 Sample wells**

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
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