Enzyme Immunoassay for Mouse Plasmin Activity
For Research Use Only

INTRODUCTION

Plasmin is a serine protease that is released as the zymogen plasminogen into the circulation system and is activated by tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA), thrombin and fibrin. Once activated, this enzyme degrades proteins found in blood plasma, primarily fibrin clots. Active plasmin in blood is rapidly inactivated by forming a covalent complex with alpha 2-antiplasmin. Decreased levels of plasmin may lead to thrombosis due to a reduced ability to dissolve clots.

PRINCIPLES OF PROCEDURE

Functionally active plasmin in samples will bind to biotinylated active site inhibitor peptide in solution in the reaction plate. Only free, active enzyme will be inhibited, not plasminogen or complexed plasmin. The inhibited plasmin will bind to capture antibody coated on the immunoassay plate. After washing, an avidin-HRP conjugate is reacted to the biotin labeled inhibited plasmin. TMB substrate is applied and is converted by HRP and can be quantified at 450 nm.

MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Component</th>
<th>Contents</th>
<th>Quantity</th>
<th>Storage</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Plate</td>
<td>Non-Binding 96 Well Coated Plate</td>
<td>1</td>
<td>4°C</td>
<td>VA38a</td>
</tr>
<tr>
<td>Immunoassay Plate</td>
<td>Anti-Mouse Plasmin Coated 96 Well Plate</td>
<td>1</td>
<td>4°C</td>
<td>VA38b</td>
</tr>
<tr>
<td>10x Wash Buffer</td>
<td>Concentrated Wash Buffer</td>
<td>50 mL</td>
<td>4°C</td>
<td>VA38c</td>
</tr>
<tr>
<td>Mouse Plasmin Standard</td>
<td>Lyophilized Standard</td>
<td>1</td>
<td>4°C</td>
<td>VA38d</td>
</tr>
<tr>
<td>Biotinylated Inhibitor</td>
<td>Lyophilized Biotinylated Inhibitor</td>
<td>1</td>
<td>4°C</td>
<td>VA38e</td>
</tr>
<tr>
<td>Avidin-HRP Conjugate</td>
<td>HRP Labeled Avidin</td>
<td>1</td>
<td>4°C</td>
<td>VA38f</td>
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<tr>
<td>TMB Substrate</td>
<td>Ready To Use TMB Substrate</td>
<td>10 mL</td>
<td>4°C</td>
<td>VA38g</td>
</tr>
</tbody>
</table>

MATERIALS NEEDED BUT NOT PROVIDED

1. Adjustable pipettes (10-1,000 µL) and disposable tips
2. Beakers, flasks, and cylinders as necessary for preparation of reagents
3. Microplate reader with 450 nm filter
4. Deionized Water
5. 1N Sulfuric Acid
6. BSA Blocking Buffer (see reagent preparation below)
7. TBS Buffer (see reagent preparation below)
STORAGE CONDITIONS

1. Store this kit and its components at 4°C until use.
2. Do not freeze.

WARNINGS AND PRECAUTIONS

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.

PROCEDURAL NOTES

1. Notes go here

SAMPLE COLLECTION AND PREPARATION

Samples of plasma, serum, cell culture media, or other biological fluids may be used directly in the assay. The assay measures active mouse plasmin in the 0.02-10 ug/mL range. Samples giving plasmin levels above 10 ug/mL should be diluted in BSA blocking buffer before use.

REAGENT PREPARATION

1. Prepare a 1x TBS Buffer: 0.1M Tris-HCl, 0.15M NaCl, pH7.4.
2. Prepare a 3% BSA Blocking Buffer: Add 3% w/v BSA to 1x TBS Buffer.
3. Dilute the 10x Wash Buffer with DI water to a 1x concentration.

STANDARD PREPARATION

Reconstitute the standard as directed on the vial to give a 25 ug/mL standard stock solution. Prepare the plasmin standard curve using the table below.

Table 1: Preparation of Standard Curve

<table>
<thead>
<tr>
<th>Standard</th>
<th>Plasmin Concentration (µg/mL)</th>
<th>3% BSA Blocking Buffer (µL)</th>
<th>Transfer Volume (µL)</th>
<th>Transfer Source</th>
<th>Final Volume (µL)</th>
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</thead>
<tbody>
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<td>10</td>
<td>600</td>
<td>400</td>
<td>Stock</td>
<td>500</td>
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<tr>
<td>S₈</td>
<td>5</td>
<td>500</td>
<td>500</td>
<td>S₀</td>
<td>600</td>
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<tr>
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<td>S₈</td>
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<tr>
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<td>500</td>
<td>500</td>
<td>S₇</td>
<td>500</td>
</tr>
<tr>
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<td>S₆</td>
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<td>500</td>
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<td>500</td>
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<tr>
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<td>500</td>
<td>500</td>
<td>S₃</td>
<td>600</td>
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<tr>
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<td>0</td>
<td>500</td>
<td></td>
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</table>
ASSAY PROCEDURE

1. Reconstitute the biotinylated inhibitor (VA38e) as directed on the vial. Add 20 µL to each well of the non-binding reaction plate (VA38a).
2. Add 100 µL of plasmin standards and unknown samples in duplicate wells. Be sure to record their positions.
3. Incubate the plate at room temperature for 30 minutes.
4. Transfer 100 µL from each well of the reaction plate to the corresponding wells of the immunoassay plate (VA38b).
5. Incubate for 30 minutes at room temperature. If possible, use an orbital shaker at 300 rpm.
6. Wash all wells three times with 1x wash buffer. Gently tap the wells dry against paper towel or kimwipes.
7. Add 2 µL of avidin-HRP conjugate to 10 mL of 3% BSA blocking buffer. Add 100 µL of this mixture to each well of the immunoassay plate.
8. Incubate for 30 minutes at room temperature. If possible, use an orbital shaker at 300 rpm.
9. Wash all wells three times with 1x wash buffer. Gently tap the wells dry against paper towel or kimwipes.
10. Add 100 µL of TMB substrate to each well and shake for 2-10. The substrate will change from clear to blue.
11. When sufficient color had developed, quench the reaction by adding 50 µL 1N sulfuric acid to each well.
12. Read the plate at 450 nm.

CALCULATIONS

1. Average the S0 standard and subtract from all standards and unknowns.
2. Plot the absorbance at 450 nm (y axis) against the standard concentrations (x axis).
3. Use the formula y=mx+b and the standards to determine unknown concentrations.

TYPICAL VALUES

Free active plasmin concentrations in plasma are low except in cases of antiplasmin deficiency or thrombolytic therapy. In house testing of normal mouse plasma yields an active plasmin concentration of 0.2 µg/mL.
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Oxford Biomedical Research, Inc.
P.O. Box 522
Oxford, MI 48371 U.S.A.

Orders: 800-692-4633
Technical Service: 248-852-8815
Fax: 248-852-4466
E-mail: info@oxfordbiomed.com

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