Human Alpha-2 Antiplasmin Activity ELISA

Catalog Number: A2A31-K01
96 Wells
For Research Use Only
v. 1.0
INTRODUCTION

This human Alpha-2-Antiplasmin Activity ELISA assay kit is for the quantitative determination of active Alpha-2-antiplasmin in human plasma.

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

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>Coated Plate</td>
<td>Plasmin coated 96-well plate</td>
<td>1 plate</td>
<td>4°C</td>
<td>VA10a</td>
</tr>
<tr>
<td>Standard</td>
<td>Human α-2-antiplasmin activity standard</td>
<td>1 vial</td>
<td>4°C</td>
<td>VA10b</td>
</tr>
<tr>
<td>Primary Antibody</td>
<td>Anti-human antiplasmin antibody</td>
<td>1 vial</td>
<td>4°C</td>
<td>VA10c</td>
</tr>
<tr>
<td>Wash Buffer</td>
<td>10x solution for washing plate</td>
<td>50 mL</td>
<td>4°C</td>
<td>VA10d</td>
</tr>
<tr>
<td>Secondary Antibody</td>
<td>HRP conjugated antibody</td>
<td>1 vial</td>
<td>4°C</td>
<td>VA10e</td>
</tr>
<tr>
<td>Substrate</td>
<td>TMB substrate</td>
<td>10 mL</td>
<td>4°C</td>
<td>VA10f</td>
</tr>
</tbody>
</table>

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. 1N H₂SO₄
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

This assay has been validated for use with samples of human plasma in citrate anticoagulant and human 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 –20ºC or colder for extended storage.

REAGENT PREPARATION

1. Dilute the 50 mL of 10x Wash Buffer concentrate to 1x with 450 mL DI water prior to use.
2. Prepare 100 mL of TBS Buffer: 0.1 M Tris-HCL, 0.15 M NaCl, pH 7.4
3. Prepare 20 mL of 3% BSA Blocking Buffer: 3% BSA in TBS Buffer

STANDARD PREPARATION

Reconstitute the Standard as directed on the vial to give a 10 ng/mL Standard Stock Solution. Do not prepare the Standards until you are ready to apply them to the plate.

Table 1: Preparation of Standard Curve

<table>
<thead>
<tr>
<th>Standard</th>
<th>α-2-Antiplasmin Concentration (ng/mL)</th>
<th>Blocking Buffer (µL)</th>
<th>Transfer Volume (µL)</th>
<th>Transfer Source</th>
<th>Final Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₉</td>
<td>10</td>
<td>0</td>
<td>100</td>
<td>Stock Vial</td>
<td>500</td>
</tr>
<tr>
<td>S₈</td>
<td>5</td>
<td>500</td>
<td>500</td>
<td>S₉</td>
<td>500</td>
</tr>
<tr>
<td>S₇</td>
<td>2.5</td>
<td>500</td>
<td>500</td>
<td>S₈</td>
<td>600</td>
</tr>
<tr>
<td>S₆</td>
<td>1</td>
<td>600</td>
<td>400</td>
<td>S₇</td>
<td>500</td>
</tr>
<tr>
<td>S₅</td>
<td>0.5</td>
<td>500</td>
<td>500</td>
<td>S₆</td>
<td>500</td>
</tr>
<tr>
<td>S₄</td>
<td>0.25</td>
<td>500</td>
<td>500</td>
<td>S₅</td>
<td>600</td>
</tr>
<tr>
<td>S₃</td>
<td>0.1</td>
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<td>400</td>
<td>S₄</td>
<td>600</td>
</tr>
<tr>
<td>S₂</td>
<td>0.05</td>
<td>500</td>
<td>500</td>
<td>S₃</td>
<td>500</td>
</tr>
<tr>
<td>S₁</td>
<td>0.025</td>
<td>500</td>
<td>500</td>
<td>S₂</td>
<td>1000</td>
</tr>
</tbody>
</table>

ASSAY PROCEDURE

1. Add 100 µl of the Standards and unknowns to the well in duplicate. If the unknown is thought to have high antiplasmin levels, dilutions may be made in 3% BSA Blocking Buffer. For a suggested plate layout, see Scheme I on the following page.
2. Shake the plate at 300 rpm for 30 minutes at room temperature.
3. Wash the plate three times with 300 µL of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
4. Reconstitute the Primary Antibody as directed on the vial and agitate gently to completely dissolve contents. Add 100 µl to each well.
5. Shake the plate at 300 rpm for 30 minutes at room temperature.
6. Wash the plate three times with 300 µL of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
7. Dilute the Secondary Antibody in 3% BSA Blocking Buffer as directed on the vial and add 100 µl to each well.
8. Shake the plate at 300 rpm for 30 minutes room temperature.
9. Wash the plate three times with 300 µL of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
10. Add 100 µl of TMB Substrate to each well.
11. Shake the plate at 300 rpm for 5-20 minutes.
12. Stop the reaction with 50 µl of 1N H₂SO₄ and rea...

For further information about this kit, its application or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

Product Developed and Manufactured in the USA