Alveomucin ELISA Kit

Catalog Number KA4026
96 assays
Version: 01

Intended for research use only
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Introduction

Intended Use

A solid-phase enzyme immunoassay for the quantitative determination of alveomucin in biological fluids. This kit is designed for measurement of alveomucin in biological fluids. The kit contains reagents sufficient for 96 determinations and allows to analyze 42 unknown samples in duplicates.

Background

Alveomucin (AM) or mucin antigen 3EG5 is produced by alveolocytes of type 2. Elevated serum levels of AM were found in patients with interstitial lung diseases (ILD), the degree of elevation correlating to the severity of clinical symptoms. AM determination may be of use for ILD diagnostics and monitoring.

Principle of the Assay

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal to human alveomucin-antibodies. Antigen from the specimen is captured by the antibodies coated onto the microwell surface. Unbound material is removed by washing procedure. Second antibodies – murine monoclonal to human alveomucin, labelled with peroxidase enzyme, are then added into the microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.
**General Information**

**Materials Supplied**

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveomucin EIA strips: polystyrene microwells coated with murine monoclonal to human alveomucin</td>
<td>96 (8x12) wells</td>
</tr>
<tr>
<td>Calibrator set (1-5): The set contains 5 calibrators: 0; 25; 50; 100; 200 U/ml. human alveomucin diluted in phosphate buffered of preselected horses serum, casein solution, preservative – 0,1% phenol; also contains blue dye</td>
<td>0.8 ml x 5</td>
</tr>
<tr>
<td>Control serum: dilution of preselected human serum, with high content of alveomucin with casein solution; preservative – 0,1% phenol, colourless</td>
<td>0.8 ml</td>
</tr>
<tr>
<td>Conjugate: aqueous solution of murine monoclonal to human alveomucin coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0,1% phenol as preservative and red dye</td>
<td>11 ml</td>
</tr>
<tr>
<td>EIA Sample buffer: saline ; contains blue dye</td>
<td>11 ml</td>
</tr>
<tr>
<td>Substrate solution: ready-to-use single-component tetramethylbenzidine (TMB) solution</td>
<td>11 ml</td>
</tr>
<tr>
<td>Washing solution concentrate 21x: aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative</td>
<td>22 ml</td>
</tr>
<tr>
<td>Stop solution: 5,0% vol/vol solution of sulphuric acid</td>
<td>11 ml</td>
</tr>
<tr>
<td>Plate sealing tape</td>
<td>2 slides</td>
</tr>
</tbody>
</table>
**Storage Instruction**

Store the whole kit at +2…+8 °C upon receipt until the expiration date. After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

**Materials Required but Not Supplied**

- Distilled or deionized water;
- Automatic or semiautomatic multichannel micropipettes, 100–250 µl, is useful but not essential;
- Calibrated micropipettes with variable volume, range volume 25–250 µl;
- Microtiter plate shaker. Shaking should be medium to vigorous. Longitudinal shaking approximately 200 strokes/min, oscillations 600–800/min
- Calibrated microplate photometer with 450 nm wavelength and OD measuring range 0–3.0

**Precautions for Use**

- For professional use only.
- This kit is intended for research only.
- INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are not present in the reagents of this kit. All human products, including human samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.
- Avoid contact with stop solution containing 5,0% H₂SO₄ . It may cause skin irritation and burns.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.
- Do not use the kit beyond the expiration date.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.
- Do not mix reagents from different lots.
- Replace caps on reagents immediately. Do not swap caps.
- Do not pipette reagents by mouth.
- Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.
Assay Protocol

Reagent Preparation

✓ All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18…+25 °C) before use.
✓ All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
✓ It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
✓ Prepare washing solution from the Washing solution concentrate 21x by 21 dilutions in distilled water.

Sample Preparation

✓ Specimen Collection and Storage
This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.
Specimens may be stored for up to 48 hours at +2…+8 °C before testing. For a longer storage, the specimens should be frozen at -20 °C or lower. Repeated freezing/ thawing should be avoided.

✓ Specimen Processing

<table>
<thead>
<tr>
<th>Material type</th>
<th>Notes on material collection, storage and handling</th>
<th>EIA buffer into the well, µl</th>
<th>Sample into the well, µl</th>
<th>Calculation factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood serum or plasma</td>
<td>Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.</td>
<td>50</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>bronchoalveolar fluid</td>
<td>Turbid samples should give incorrect measurement results and should be treated by centrifugation before testing.</td>
<td>50</td>
<td>50</td>
<td>1</td>
</tr>
</tbody>
</table>

✓ Quality Control

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.
The test must be performed exactly as per the manufacturer’s instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.
Assay Procedure

✓ Procedure Note

It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

1. Put the desired number of microstrips into the frame; allocate 12 wells for the calibrators (1-5) and control serum and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.
2. If suggested analyte concentration in the sample exceeds the highest calibrator, additionally dilute this sample accordingly, using EIA buffer. Use of other buffers or reagents for sample dilution may lead to incorrect measurement.
3. Pipet 50 µl of EIA Sample buffer into each well.
4. Pipet 50 µl of calibrators (1-5) and control samples Control into allocated wells. For testing of serum or plasma pipet 50 µl of the unknown sample into the allocated wells. See “Sample processing” for the volumes of other materials. Pipetting should be made within 3 minutes, to ensure an uniform incubation time for all samples. Carefully mix the contents of the wells by short horizontal rotating of the plate for 5–7 seconds and cover the wells by plate adhesive tape (included into the kit).
5. Incubate 30 minutes at +18...+25 °C and continuous shaking at 600-800 rpm
6. Prepare washing solution by 21x dilution of washing solution concentrate 21x by distilled water. Minimal quantity of washing solution should be 250 µl per well. Wash strips 3 times
7. Dispense 100 µl of Conjugate into the wells. Cover the wells by plate adhesive tape.
8. Incubate 30 minutes at +18...+25 °C and continuous shaking at 600–800 rpm
9. Wash the strips 5 times.
10. Dispense 100 µl of Substrate solution into the wells
11. Incubate 10-20 minutes at +18...+25 °C
12. Dispense 100 µl of Stop solution into the wells.
13. Measure OD (optical density) at 450 nm.
14. Set photometer blank on first calibrator
15. Apply point-by-point method for data reduction. Use Calculation factor listed in “Sample processing” to calculate analyte concentration in different material types.
Data Analysis

Calculation of Results

✓ Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.
✓ Plot a calibration curve on graph paper: OD versus alveomucin concentration.
✓ Determine the corresponding concentration of alveomucin in unknown samples from the calibration curve.
  Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.
✓ Below is presented a typical example of a standard curve. Not for calculations!

<table>
<thead>
<tr>
<th>Calibrators</th>
<th>Value</th>
<th>Absorbance Units (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 U/ml</td>
<td>0.15</td>
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<tr>
<td>2</td>
<td>25 U/ml</td>
<td>0.49</td>
</tr>
<tr>
<td>3</td>
<td>50 U/ml</td>
<td>0.76</td>
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<tr>
<td>4</td>
<td>100 U/ml</td>
<td>1.24</td>
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<tr>
<td>5</td>
<td>200 U/ml</td>
<td>2.16</td>
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</table>
Expected Values

Each laboratory should establish its own normal range for Alveomucin. The following normal range is recommended (see below). NOTE: the individuals that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive individuals should be treated with depleting adsorbents before assaying.

<table>
<thead>
<tr>
<th>Sex, age</th>
<th>Units, U/ml</th>
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<tr>
<td></td>
<td>Lower limit</td>
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<tr>
<td>Healthy donors</td>
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<td>Pregnancy:</td>
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<td>1st trimester</td>
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<td>2nd trimester</td>
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<td>3rd trimester</td>
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<td>Lactation</td>
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Performance Characteristics

- Analytical sensitivity
  Sensitivity of the assay was assessed as being 20 U/ml.

- Linearity
  Linearity was checked by assaying dilution series of 5 samples with different alveomucin concentrations. Linearity percentages obtained ranged within 90 to 110%.

- Recovery
  Recovery was estimated by assaying 5 mixed samples with known alveomucin concentrations. The recovery percentages ranged from 90 to 110%.
Resources

References

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