Anti-Elastase EIA

This kit is a solid phase enzyme immunoassay employing native human Elastase for the quantitative detection of antibodies against Elastase in human serum.

For Research Use Only. Not For Use In Diagnostic Procedures.

35-ELAHU-E01 replaces 35-3305

Catalog Number: 35-ELAHU-E01
Size: 96 wells
Version: 002 : 2007-08-28 – ALPCO 08/21/2012
1. Intended Use

_Anti-Elastase EIA_ is a solid phase enzyme immunoassay employing native human Elastase for the quantitative and qualitative detection of antibodies against Elastase in human serum.

2. Application and Principle of the Assay

Elastase is a serine protease with a homology of 54% to proteinase 3, occurring mainly in polymorph-nuclear neutrophilic granulocytes (PMN), in macrophages and endothelial cells. The proteolytic activity of Elastase secreted by neutrophils is responsible for the dismantling of proteoglycans. Furthermore, Elastase participates decisively in tissue destruction connected with emphysemas and rheumatoid arthritis.

Antibodies against Elastase belong to the group of anti-neutrophil cytoplasmic antibodies (ANCA) which are directed against cytoplasmic components of neutrophilic granulocytes and monocytes. For the detection of ANCAs indirect immunofluorescence test on ethanol-fixed neutrophils has been the established method so far. It became apparent that some ANCAs create a cytoplasmic fluorescence pattern (thus called cANCA) while others create a perinuclear pattern (the pANCA). As both patterns may cover multiple antigens, immunofluorescence does not suffice for a satisfying differential diagnosis of vasculitis; thus each IFT should be verified with specific ELISA tests.

Proteinase 3 (PR3) and Myeloperoxidase (MPO) have been identified as the major cANCA and pANCA antigens, respectively, but other cellular components like Lactoferrin, Cathepsin G and Elastase cause perinuclear staining too and therefore are included into the group of pANCAs.

Detection of ANCAs is a useful laboratory test for certain small vessel vasculitides and some non-vasculitic syndromes, such as inflammatory bowel disease (IBD). Antibodies against MPO correlate with idiopathic or vasculitis associated necrotizing crescentic glomerulonephritis. They are found frequently in 70% of individuals with microscopic polyangiitis as well as 5-50% of individuals with Churg-Strauss syndrome. Autoantibodies to PR3 are a specific serological marker for Wegener’s granulomatosis (WG).

Autoantibodies against Elastase are generally associated with inflammatory rheumatic disorders, e.g. rheumatoid arthritis and vasculitis.

**Principle of the test**

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the sample.
3. Kit Contents

To be reconstituted:

5x Sample Buffer  1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
                   Containing: Tris, NaCl, BSA, sodium azide <0.1% (preservative)

50x Wash Buffer   1 vial, 20 ml - 50x concentrated (capped white: green solution)
                   Containing: Tris, NaCl, Tween, sodium azide <0.1% (preservative)

Ready to use:

Negative Control 1 vial, 1.5 ml (capped green: colorless solution)
                   Containing: Human serum (diluted), sodium azide <0.1% (preservative)

Positive Control 1 vial, 1.5 ml (capped red: yellow solution)
                   Containing: Human serum (diluted), sodium azide <0.1% (preservative)

Cut-off Control  1 vial, 1.5 ml (capped blue: yellow solution)
                   Containing: Human serum (diluted), sodium azide <0.1% (preservative)

Calibrators      6 vials, 1.5 ml each  0, 3, 10, 30, 100, 300 U/ml
                   (color increasing with concentration: yellow solutions)
                   Containing: Human serum (diluted), sodium azide <0.1% (preservative)

Conjugate        1 vial, 15 ml IgG (capped blue: blue solution)
                   Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase

TMB Substrate    1 vial, 15 ml (capped black)
                   Containing: Stabilized TMB/H2O2

Stop Solution    1 vial, 15 ml (capped white: colorless solution)
                   Containing: 1M Hydrochloric Acid

Microtiterplate  12x8 well strips with breakaway microwells
                   Coating see paragraph 1

Material required but not provided:
Microliter plate reader with a 450 nm reading filter and optional a 620 nm reference filter (600-690
nm). Glassware, test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200,500, 1000 µl
or adjustable multipipette (100-1000 ml). Microplate washing device (300 µl repeating or multichannel
pipette or automated system), absorbent paper. Our tests are designed to be used with purified water
according to the definition of the United States Pharmacopeia (USP 26-NF21) and the European
Pharmacopeia (Eur.Ph. 4th ed.).

4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared,
reconstituted solutions are stable for 1 month at 4°C/39°F, at least. Reagents and the microplate
shall be used within the expiry date indicated on each component only. Avoid intense exposure
of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal
tightly.
6. Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Do not use icteric, lipemic, hemolyzed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry, and empty tubes. After separation, the serum samples should be used immediately, respectively stored tightly closed at 2-8°C/35-45°F up to three days, or frozen at -20°C/-4°F for longer periods. Blood withdrawal must follow national requirements.

5. Precautions of Use

5.1 Health hazard data

*This product is for Research Use Only. Not For Use In Diagnostic Procedures.*

Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

**Recommendations and precautions**

This kit contains potentially hazardous components. Though kit reagents are not classified as being irritating to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves. WARNING: Calibrators, Controls, and Buffers contain sodium azide (NaN₃) as a preservative. NaN₃ may be toxic if ingested or absorbed by the skin or eyes. NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines. Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth. All human source material used in kit reagents (e.g. controls, standards) has been tested by approved methods and found negative for HbsAg, Hepatitis C, and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus, handle kit controls, standards, and samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results. Allow all components to reach room temperature (20-26°C/68-89.6°F) before use mix well and follow the recommended incubation scheme for an optimum performance of the test. **Incubation: We recommend test performance at 30°C/86°F for automated systems.** Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior. Never expose components to temperatures higher than 37°C/98.6°F.
7. Assay Procedure

7.1 Preparations prior to pipetting

Dilute concentrated reagents:
Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).
Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Samples
Dilute serum samples 1:101 with sample buffer (1x)
e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well!

Washing
Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells.
e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:
Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:
Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean absorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates
Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

7.2 Work flow

We recommend pipetting samples and calibrators in duplicate. Cut-off calibrator should be used for qualitative testing only.

Pipette 100 µl of each diluted sample into the designated microwells.
Pipette 100 µl calibrators OR cut-off control and negative and positive controls into the designated wells.
Incubate for 30 minutes at 20-32°C/68-89.6°F.
Wash 3x with 300 µl washing buffer (diluted 1:50).
Pipette 100 µl conjugate into each well.
Incubate for 30 minutes at 20-32°C/68-89.6°F.
Wash 3x with 300 µl washing buffer (diluted 1:50).
Pipette 100 µl TMB substrate into each well.
Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
Incubate 5 minutes minimum.
Agitate plate carefully for 5 sec.
Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.
8. Quantitative and Qualitative Interpretation

For quantitative interpretation, establish the standard curve by plotting the optical density (O.D.) of each calibrator (y-axis) with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the O.D. of each sample, read the corresponding antibody concentrations expressed in U/ml.

<table>
<thead>
<tr>
<th>Normal Range</th>
<th>Equivocal Range</th>
<th>Positive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 12 U/ml</td>
<td>12-18 U/ml</td>
<td>&gt; 18 U/ml</td>
</tr>
</tbody>
</table>

**Example of a standard curve**

We recommend pipetting calibrators in parallel for each run.

<table>
<thead>
<tr>
<th>Calibrators</th>
<th>O.D. 450/620 nm</th>
<th>CV % (Variation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG 0 U/ml</td>
<td>0.032</td>
<td>2.8</td>
</tr>
<tr>
<td>IgG 3 U/ml</td>
<td>0.152</td>
<td>2.6</td>
</tr>
<tr>
<td>IgG 10 U/ml</td>
<td>0.281</td>
<td>1.2</td>
</tr>
<tr>
<td>IgG 30 U/ml</td>
<td>0.646</td>
<td>2.4</td>
</tr>
<tr>
<td>IgG 100 U/ml</td>
<td>1.214</td>
<td>1.7</td>
</tr>
<tr>
<td>IgG 300 U/ml</td>
<td>2.104</td>
<td>1.6</td>
</tr>
</tbody>
</table>

**Example of calculation**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Replicate (OD)</th>
<th>Mean (OD)</th>
<th>Result (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P01</td>
<td>0.658/0.669</td>
<td>0.664</td>
<td>30.6</td>
</tr>
<tr>
<td>P02</td>
<td>1.156/1.148</td>
<td>1.152</td>
<td>86.2</td>
</tr>
</tbody>
</table>

For lot specific data, see enclosed quality control leaflet. Laboratories might perform an in-house Quality Control by using their own controls and/or internal pooled sera, as foreseen by EU regulations. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and sample population according to its own established procedures.

For qualitative interpretation, read the optical density of the cut-off calibrator and the samples.

Compare the sample OD with the cut-off calibrator OD. We recommend considering sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

**Negative:** OD sample < 0.8 x OD cut-off

**Equivocal:** 0.8 x OD cut-off ≤ OD sample ≤ 1.2 x OD cut-off

**Positive:** OD sample > 1.2 x OD cut-off

9. Technical Data

- **Sample material:** serum
- **Sample volume:** 10 µl of sample diluted 1:101 with 1x sample buffer
- **Total incubation time:** 90 minutes at 20-32°C/68-89.6°F
- **Calibration range:** 0-300 U/ml
- **Analytical sensitivity:** 1.0 U/ml
- **Storage:** at 2-8°C/35-46°F use original vials, only
- **Number of determinations:** 96 tests
10. Performance Data

10.1 Analytical Sensitivity
Testing sample buffer 30 times on this kit gave an analytical sensitivity of 1.0 U/ml.

10.2 Specificity and sensitivity
The microplate is coated with highly purified native Elastase. No crossreactivities to other autoantigens have been found. For sensitivity and specificity there are currently no valid data for anti-Elastase antibodies available.

10.3 Linearity
Chose sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Dilution Factor</th>
<th>Measured Concentration (U/ml)</th>
<th>Expected Concentration (U/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/100</td>
<td>226.0</td>
<td>224.0</td>
<td>100.9</td>
</tr>
<tr>
<td></td>
<td>1/200</td>
<td>113.0</td>
<td>112.0</td>
<td>100.9</td>
</tr>
<tr>
<td></td>
<td>1/400</td>
<td>54.0</td>
<td>56.0</td>
<td>96.4</td>
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<td></td>
<td>1/800</td>
<td>26.0</td>
<td>28.0</td>
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<td>2</td>
<td>1/100</td>
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<td>1/800</td>
<td>22.0</td>
<td>23.0</td>
<td>95.7</td>
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</table>

10.4 Precision
To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

<table>
<thead>
<tr>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
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</thead>
<tbody>
<tr>
<td>Sample No.</td>
<td>Mean (U/ml)</td>
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<tr>
<td>1</td>
<td>130.0</td>
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<tr>
<td>2</td>
<td>95.0</td>
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<tr>
<td>3</td>
<td>63.0</td>
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</table>

10.5 Calibration
Due to the lack of international reference calibration this Anti-Elastase kit is calibrated in arbitrary units (U/ml).
11. Literature


Pipetting Scheme
We suggest pipetting calibrators, controls, and samples as follows:
For quantitative interpretation use calibrators to establish a standard curve.
For qualitative interpretation use cut-off control.

- For quantitative interpretation use calibrators to establish a standard curve
- For qualitative interpretation use cut-off control

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
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<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>A</td>
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<td>CalE</td>
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<td></td>
<td></td>
<td></td>
<td>NC</td>
<td>S2</td>
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<tr>
<td>B</td>
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<td>CalE</td>
<td>S1</td>
<td></td>
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<tr>
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<td>CalF</td>
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<td>CC</td>
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PC: positive control  NC: negative control  CC: cut-off control  S: Sample