Lactoferrin Ab ELISA Kit

Catalog Number KA1267
96 assays
Version: 05

Intended for research use only

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Introduction

Intended Use

Enzyme immunoassay for quantitative determination of IgG auto-antibodies to Lactoferrin in human serum or plasma. The assay is intended for research use only.

Background

Anti-neutrophil cytoplasmic antibodies (ANCA) represent a group of autoantibodies directed towards cytoplasmic components of the neutrophil granulocytes and monocytes. The classical methods for the determination of ANCA are immunofluorescence tests. With these indirect immunofluorescence (IF) techniques two main patterns are distinguished: a cytoplasmic (cANCA) and a perinuclear (pANCA) type. The target antigen for 80-90% of cANCA is proteinase 3 (PR3), a serine proteinase present in primary granules; 10-20% of cANCA are directed to other proteins, such as bactericidal permeability-increasing protein (BPI). In rare cases, antibodies to elastase (4%), lysozym (2%) or cathepsin G (2%) may show a cANCA-pattern. cANCA have also been detected in different non-rheumatic diseases. Approximately 90% of pANCA positive sera contain autoantibodies directed to myeloperoxidase (MPO), which is located in the granules of neutrophil granulocytes. Antibodies to other antigens e.g. Lactoferrin, Elastase, Cathepsin-G and also Lysozyme often result in a similar pANCA pattern. These atypical pANCA occur in collagenosis and related inflammatory rheumatic diseases. Besides, different untypical variants of pANCA IF patterns – granulocyte specific antinuclear antibodies (GS-ANA) – are indistinguishable from pANCA. Therefore, a distinct interpretation and classification of the IF patterns is difficult and every positive IFANCA finding should be differentiated by ELISA techniques using the purified single antigens.

Lactoferrin (LF) is an iron-binding protein with physiological anti microbial effect and it is a non-specific antiphlogistic defence factor at mucosal surfaces. Lactoferrin occurs in secretions such as milk, tears and saliva. Lactoferrin also resides in the specific granules of polymorph nuclear neutrophil leukocytes (PMN). During active inflammatory disease, raised serum levels of Lactoferrin can be measured. Autoantibodies against Lactoferrin belong to the pANCA group. They occur in higher frequency in patients with rheumatoid vasculitis (RV), ulcerative colitis (CU) and primary sclerosing cholangitis (PSC).
A survey of documented clinical indications, the corresponding immunofluorescence patterns and target antigens is given in the following table:

<table>
<thead>
<tr>
<th>Diseases</th>
<th>IF pattern</th>
<th>Target antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systemic Vasculitis Syndromes</strong></td>
<td></td>
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<tr>
<td>Wegener’s Granulomatosis</td>
<td>c-ANCA, rare p-ANCA</td>
<td>PR3, rare MPO</td>
</tr>
<tr>
<td>Microscopic Polyangitis</td>
<td>c-ANCA, p-ANCA</td>
<td>PR3, MPO</td>
</tr>
<tr>
<td>Churg-Strauss-Snydrome</td>
<td>p-ANCA</td>
<td>MPO</td>
</tr>
<tr>
<td>Polyarteritis nodosa</td>
<td>Rare ANCA</td>
<td>Rare PR3 and MPO</td>
</tr>
<tr>
<td>Unclassified Vasculitis</td>
<td>Rare</td>
<td>No PR3 and MPO</td>
</tr>
<tr>
<td><strong>Collagen Diseases and other Rheumatic Disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>GS-ANA, p-ANCA, atypical ANCA</td>
<td>Unknown, ANA, rare MPO, Lactoferrin</td>
</tr>
<tr>
<td>SLE</td>
<td>p-ANCA</td>
<td>Rare MPO, Lactoferrin</td>
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<tr>
<td><strong>Other Diseases</strong></td>
<td></td>
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<tr>
<td>Ulcerative Colitis</td>
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<td>Cathepsin-G, Lactoferrin</td>
</tr>
</tbody>
</table>

**Principle of the Assay**

Highly purified Lactoferrin is bound to microwells. Antibodies to this antigen, if present in diluted sample, bind in the respective antigen. Washing of the microwells removes unbound serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume / Qty.</th>
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</thead>
<tbody>
<tr>
<td>Divisible microplate Consisting of 12 modules of 8 wells each. Ready to use.</td>
<td>96 (8x12) wells</td>
</tr>
<tr>
<td>Calibrators A-F (0, 6.3, 12.5, 25, 50, 100 U/ml), containing serum/buffer matrix (PBS, BSA, detergent, NaN₃ 0.09%), yellow. Ready to use.</td>
<td>1.5 ml x 2</td>
</tr>
<tr>
<td>Control positive (1) and negative (2), containing lactoferrin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN₃ 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.</td>
<td>1.5 ml x 2</td>
</tr>
<tr>
<td>Sample buffer P, containing PBS, BSA, detergent, preservative NaN₃ 0.09%, yellow, 5x conc.</td>
<td>20 ml</td>
</tr>
<tr>
<td>Enzyme conjugate containing anti-human IgG antibodies, HRP labeled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.</td>
<td>15 ml</td>
</tr>
<tr>
<td>TMB substrate; containing 3,3’, 5,5’-Tetramethylbenzidin, colorless. Ready to use.</td>
<td>15 ml</td>
</tr>
<tr>
<td>Stop solution (contains acid). Ready to use.</td>
<td>15 ml</td>
</tr>
<tr>
<td>Wash solution, containing Tris, detergent, preservative NaN₃ 0.09%; 50x conc.</td>
<td>20 ml</td>
</tr>
</tbody>
</table>

Storage Instruction

- Store the kit at 2-8°C in the dark.
- Do not expose test reagents to heat, sun or strong light during storage and usage.
- Store microplate sealed and desiccated in the clip bag provided.
- Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Solution and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

Materials Required but Not Supplied

- Microplate reader capable of endpoint measurements at 450 nm: reference filter at 620 nm.
- Data reduction software
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

Precautions for Use

- Procedural Notes
  - Do not use kit components beyond their expiration dates.
  - Do not interchange kit components from different lots and products.
  - All materials must be at room temperature (20-28°C) prior to use.
  - Prepare all reagents and samples. Once started, perform the test without interruption.
  - Double determinations may be done. By this means pipetting errors may become obvious.
  - Perform the assay steps only in the order indicated.
  - Always use fresh sample dilutions.
  - Pipette all reagents and samples into the bottom of the wells.
  - To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
  - Wash microwells thoroughly and remove the last droplets of wash solution.
  - All incubation steps must be accurately timed.
  - Do not re-use microplate wells.

- Warnings and Precautions
  - Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2 and so all human serum based reagents in this kit must be handled as through capable of transmitting infection.
  - Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
  - Avoid contact with the substrate TMB (3,3',5,5'-Tetramethyl-benzidine).
  - Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
  - Control, Calibrators, sample buffer and Wash Solution contain sodium azide (NaN₃) 0.09% as preservative. This concentration is classified as non-hazardous.
  - Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
  - During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:
    - First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
    - Personal precautions, protective equipment and emergency procedures:
    - Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette
by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

✓ Exposure controls/ personal protection: Wear protective gloves of nitrile rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.

✓ Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.

✓ For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying controls sera.
Assay Protocol

Reagent Preparation

- Wash Solution
  Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use.

- Sample Buffer
  Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionized water to a volume of 100 ml.

Sample Preparation

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum or plasma by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
5. Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
6. Testing of heat-inactivated sera is not recommended.
7. Dilute samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators/ Controls are ready to use and need not be diluted.

Assay Procedure

Prepare enough microplate modules for all calibrators/ controls and samples.
1. Pipette 100 µl of calibrators, controls and prediluted samples into the wells.
2. Incubate for 30 minutes at room temperature (20-28 °C).
3. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
4. Dispense 100 µl of enzyme conjugate into each well.
5. Incubate for 15 minutes at room temperature.
6. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
7. Dispense 100 µl of TMB substrate solution into each well.
8. Incubate for 15 minutes at room temperature.
9. Add 100 µl of stop solution to each well of the modules.
10. Incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm (reference 600-690nm) and calculate the results. The developed
colour is stable for at least 30 minutes. Read during this time.

- Validation
  Test results are valid if the optical densities at 450 nm for calibrators/controls and the results controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit. If these quality control criteria are not met the assay run is invalid and should be repeated.
Data Analysis

Calculation of Results

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of samples may then be estimated from the calibration curve by interpolation.

Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Performance Characteristics

- **Calibration**
  This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

- **Measuring range**
  The calculation range of this ELISA assay is 0-100 U/ml.

- **Expected values**
  In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 10 U/ml.

- **Interpretation of results**
  Negative:  < 10 U/ml
  Positive:  ≥ 10 U/ml

- **Linearity**
  Samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and upper/ lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.
Sample Dilution Observe [U/ml] Expected [U/ml] O/E [%]  
1 1:100 97.4 79.4 100  
- 1:200 38.9 39.7 98  
- 1:400 19.4 19.9 97  
- 1:800 10.0 9.9 101  
- 1:1600 4.5 5.0 90  
2 1:100 58.5 58.5 100  
- 1:200 29.5 29.3 101  
- 1:400 14.8 14.6 101  
- 1:800 7.9 7.3 108  
- 1:1600 3.3 3.7 89  

- Limit of detection
Functional sensitivity was determined to be: 0.5 U/ml.

- Interfering Substances
No interference has been observed with haemolytic (up to 1000 mg/dL) or lipemic (up to 3 g/dL triglycerides) sera or plasma, or bilirubin (up to 40 mg/dL) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

- Reproducibility
Intra-Assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean [U/ml]</th>
<th>CV [%]</th>
<th>Sample</th>
<th>Mean [U/ml]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.8</td>
<td>4.3</td>
<td>1</td>
<td>10.6</td>
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<td>2</td>
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<td>29.3</td>
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<td>3</td>
<td>60.4</td>
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<td>3</td>
<td>61.4</td>
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- **Study results**

<table>
<thead>
<tr>
<th>Study population</th>
<th>IFA</th>
<th>n</th>
<th>n Pos</th>
<th>%</th>
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<tbody>
<tr>
<td>ANCA vasculitis</td>
<td>pos</td>
<td>54</td>
<td>12</td>
<td>22.2</td>
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<tr>
<td>Other conditions</td>
<td>pos</td>
<td>35</td>
<td>12</td>
<td>34.3</td>
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<td>Non-ANCA vasculitis</td>
<td>pos</td>
<td>13</td>
<td>4</td>
<td>30.8</td>
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<td>Healthy controls</td>
<td>neg</td>
<td>120</td>
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<td>Non-rheumatological</td>
<td>neg</td>
<td>72</td>
<td>11</td>
<td>15.3</td>
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<tr>
<td>Non-ANCA vasculitis</td>
<td>neg</td>
<td>42</td>
<td>4</td>
<td>9.5</td>
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Resources

References


<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Calibrators A</th>
<th>Calibrators B</th>
<th>Calibrators C</th>
<th>Calibrators D</th>
<th>Calibrators E</th>
<th>Calibrators F</th>
<th>Positive Control</th>
<th>Negative Control</th>
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<tbody>
<tr>
<td>Column A</td>
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