dsDNA IgG ELISA Kit

Catalog Number KA1100
96 assays
Version: 03

Intended for research use only
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Introduction

Intended Use

dsDNA IgG ELISA Kit is an enzyme immunoassay for qualitative determination of IgG Autoantibodies to ds-DNA in human serum or plasma

Background

Connective tissue diseases (CTD) are a group of autoimmune disorders which are characterized by presence of antinuclear antibodies (ANA) in the blood of patients. ANA are a specific class of autoantibodies that have the capability of binding and destroying certain structures within the nucleus of the cells. These antibodies are involved in the disease pathogenesis, and they also constitute the basis for diagnosis and treatment of CTD. ANA have been categorized into two main groups:

1. Autoantibodies to DNA and histones
2. Autoantibodies to extractable nuclear antigens (ENA): Sm, ribonucleoproteins (RNP), SSA/Ro, SSB/La, Scl-70, Jo-1 and PM1

Autoantibodies to DNA and histones include antibodies against single and double stranded DNA (ssDNA and dsDNA). Significant levels of anti-dsDNA antibodies are considered to be confirmatory in the diagnosis of systemic lupus erythematosus (SLE). Anti-histone antibodies are indicative of drug-induced lupus. Besides DNA and histones, autoantibodies may also target other nuclear antigens. These nuclear antigens were named extractable nuclear antigens (ENA), as originally they were extracted from the nuclei with saline solution. Autoantibodies to Smith antigen (Sm) which is also considered to be highly specific for SLE were the first anti-ENA detected. Thereafter, further subtypes of ENA i.e. ribonucleoproteins (RNP), Sjögren antigen A or B (SSA/Ro or SSB/La), Scl-70, Jo-1 and PM1 were identified.

Although most of these ENA are disease specific, a significant overlap exists. Sensitivity and specificity may also vary depending upon the type of underlying CTD. Presence of autoantibodies in the sera of patients constitutes one of the criteria used for diagnosis of CTD. Together with the clinical diagnosis ANA subtyping helps in identifying a specific CTD. Indirect immunofluorescence tests (IF) and enzyme immunoassays (ELISA) are commonly used for ANA detection in day to day practice. Initially, screening is carried out by IF-ANA or a generic ELISA which detects ANA of a broad specificity similar to IF-ANA. If positive, more specific tests are performed based on clinical findings and the IF-ANA staining pattern.

These antigen specific ELISA assays react with single autoantigens e.g. dsDNA, SS-A/Ro, SS-B/La, Scl-70, Sm, Sm/RNP etc. Autoantibodies to dsDNA are specific and diagnostic for SLE and levels are elevated during active disease. Recently published ACR Guidelines for Screening, Treatment, and Management of Lupus Nephritis recommend the testing of antibodies to dsDNA for monitoring of lupus nephritis, ranging from monthly intervals in pregnant patients with active glomerulonephritis at onset of treatment to every three months in patients with active nephritis at onset of treatment or pregnant patients with previous but not current nephritis, up to six-monthly testing in patients with previous active nephritis or no prior or current nephritis.
SLE-Patients without antibodies against dsDNA often produce antibodies against ssDNA. Similarly anti-Sm is highly specific for SLE but is present in only 10% to 30% of SLE cases. Antibodies against dsDNA, histones, the 70 kD protein of the U1-snRNP complex (RNP70) and anti Sm are closely associated with SLE. Anti-SSA/Ro and anti-SSB/La antibodies are indicative for Sjögren's syndrome, but can also be found in up to 30% cases of SLE with cutaneous involvement. Anti-SS-A/Ro antibodies pass the placenta and may cause the development of SLE in neonates. Anti-SSA/Ro antibodies are almost always present in sera of mothers with babies with neonatal lupus syndrome and with complete congenital heart block. Antinucleolar antibodies are a group of autoantibodies which give a nucleolar IF-staining pattern. Most common of these are anti-PM-Scl, anti-RNA polymerase I-III and anti-U3-RNP. They are found in scleroderma and polymyositis (PM). Antibodies against RNP and the complex RNP/Sm are linked to mixed connective tissue disease (MCTD, Sharp syndrome) and to SLE. Serologically MCTD is characterized by the presence of autoantibodies directed against the 70 kD protein of the U1-snRNP-complex. Up to 100% of MCTD patients manifest high titers of Anti-RNP-70 antibodies.

Autoantibody prevalence to (values in %)

<table>
<thead>
<tr>
<th>Diseases</th>
<th>dsDNA</th>
<th>ssDNA</th>
<th>Histone</th>
<th>SS-A</th>
<th>SS-B</th>
<th>Sm</th>
<th>RNP/Sm</th>
<th>Scl-70</th>
<th>Jo-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic lupus Erythematous (SLE)</td>
<td>&gt; 90</td>
<td>&gt; 90</td>
<td>30-50</td>
<td>10-30</td>
<td>30-50</td>
<td>10-30</td>
<td>10-30</td>
<td>10-30</td>
<td></td>
</tr>
<tr>
<td>Drug-induced Lupus (DIL)</td>
<td>30-50</td>
<td>50-90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sharp-syndrome / mixed connective tissue disease</td>
<td>10-30</td>
<td>10-30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt; 90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>10-30</td>
<td>30-50</td>
<td>30-50</td>
<td>10-30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
<td>10-30</td>
<td>10-30</td>
<td>&gt; 90</td>
<td>&gt; 90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scleroderma</td>
<td>10-30</td>
<td>10-30</td>
<td>10-30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt; 90</td>
</tr>
<tr>
<td>Photosensitive dermatitis, dermatomyositis</td>
<td>10-30</td>
<td>10-30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50-90</td>
</tr>
</tbody>
</table>

**Principle of the Assay**

Human recombinant double-stranded DNA (dsDNA) is bound to microwells. Antibodies against the coated antigen, if present in diluted sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects 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 color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color 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>Component</th>
<th>Amount</th>
</tr>
</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>Calibrator A-F (0; 12.5; 25; 50; 100; and 200 IU/ml), containing dsDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN₃ &lt;0.09%), yellow. Ready to use.</td>
<td>6 x 1.5 ml</td>
</tr>
<tr>
<td>Control positive (1) and negative (2), containing dsDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN₃ &lt;0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.</td>
<td>2 x 1.5 ml</td>
</tr>
<tr>
<td>Sample Buffer PD, containing PBS, BSA, detergent, preservative NaN₃ 0.09%, yellow, concentrate (5x)</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 an 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; optional: 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 deionized water
- Measuring cylinder for 1000 ml and 100 ml
Plastic container for storage of the wash solution

The 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**

- **Warning and Precautions**
  - All reagents of this kit are intended for research use only.
  - 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 though 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.
  - Controls, calibrator, sample buffer and wash solution contain sodium azide (NaNO₃) 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 control sera.

- **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.
• Pepette 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.
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 PD Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionized water to a final volume of 100 ml.

Sample Preparation

✓ Specimen Collection, Storage and Handling
   • Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
   • Allow blood to clot and separate the serum by centrifugation.
   • Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
   • Specimens may be refrigerated at 2-8° C for up to five days or stored at -20° C up to six months.
   • Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of autoantibody activity.
   • Testing of heat-inactivated sera is not recommended.

✓ Preparation of Samples
   • Dilute all 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. Pipet 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 600-690 nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read optical densities during this time.

✓ Validation

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit. If there 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 choice.

Performance Characteristics

✓ Calibration

This assay system is calibrated against the internationally reference preparation WHO Wo/80 for human anti-dsDNA IgG antibodies as 200 IU/ml.

✓ Measuring range: The calculation range of this ELISA assay is 0-200 IU/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 20 IU/ml

✓ Interpretation of results

Negative: < 20 IU/ml
Positive: > 20 IU/ml

✓ Linearity

Samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay. Activity for each dilution was calculated by means of SMC Tecnology.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed IU/ml</th>
<th>Expected IU/ml</th>
<th>O/E [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:100</td>
<td>104.2</td>
<td>104.2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1:200</td>
<td>50.6</td>
<td>52.1</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>1:400</td>
<td>24.9</td>
<td>26.1</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>1:800</td>
<td>13.2</td>
<td>13.0</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>1:100</td>
<td>135.3</td>
<td>135.3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1:200</td>
<td>68.9</td>
<td>67.7</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>1:400</td>
<td>39.2</td>
<td>39.8</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>1:800</td>
<td>18.2</td>
<td>16.9</td>
<td>108</td>
</tr>
</tbody>
</table>
✓ Limit of detection

Functional sensitivity was determined to be: 1 IU/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, Heparin). 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.

Inter-Assay precision. Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different run. Results for run-to-run precision are shown in the table below.

| Intra-Assay IgG | | Inter-Assay IgG |
|-----------------|-----------------|
| Sample | Mean [IU/ml] | CV [%] | Sample | Mean [IU/ml] | CV [%] |
| 1 | 26.0 | 4.5 | 1 | 29.0 | 12.4 |
| 2 | 61.0 | 3.1 | 2 | 68.0 | 7.3 |
| 3 | 114.0 | 6.4 | 3 | 138.0 | 5.2 |
References


15. Putova I, Dostal C, Becvar R. Prevalence of antinucleosome antibodies by enzyme-linked immunosorbent


## Plate Layout

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample 1</td>
<td>Sample 2</td>
<td>Sample 3</td>
<td>Calibrators A</td>
<td>Calibrators B</td>
<td>Calibrators C</td>
<td>Calibrators D</td>
<td>Calibrators E</td>
<td>Calibrators F</td>
<td>Positive controls</td>
<td>Negative controls</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A Calibrators</td>
<td>B Calibrators</td>
<td>C Calibrators</td>
<td>D Calibrators</td>
<td>E Calibrators</td>
<td>F Calibrators</td>
<td>G Positive controls</td>
<td>H Negative controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>