Rheumatoid Factor Ab ELISA Kit

Catalog Number KA1442
96 assays
Version: 03

Intended for research use only
Introduction

Intended Use

Enzyme Immunoassay for quantitative determination of IgG, IgM and IgA Rheumatoid Factors in human serum or plasma.

Background

The presence of IgM rheumatoid factor (RF) in the serum is the sole serological indicator included in the ACR list of criteria for the diagnosis of RA. RFs are a subset of antiglobulins directed against the Fc region of IgG. In this definition we do not include antibodies to the IgG Fab region and pepsin agglutinators, directed against neoantigens on IgG exposed by pepsin cleavage. It is claimed that the majority of antiglobulin activity in normal serum is Fab-specific, whereas antiglobulin from RA patients is mostly Fc-specific. RFs are present in the serum of 75-80% of patients with RA at some time during the disease course. However, RFs are also found in the serum of patients with infectious and autoimmune diseases, hyperglobulinemia, B-cell lymphoproliferative disorders and in the aged population. This suggests that RF may be a finding associated with B-cell hyperactivity.

Rheumatoid factors which have been found among the IgM, IgG and IgA classes of immunoglobulins, reacting only with xenogeneic Fc are not autoantibodies and are unlikely to be of pathological significance. However, RFs can bind IgG from many species, including autologous IgG, when immobilised on surfaces. Autologous binding is of higher affinity than xenogeneic binding. The here presented test systems for the determination of rheumatoid factors uses only human Fc fragments as coated antigen.

It is generally considered that high titers of RF are associated with more severe disease and the presence of extra-articular features and rheumatoid nodules. This conclusion may depend on the disease duration. Serum IgM RF may precede the onset of RA by several years. A high titer of RF in non-RA individuals is associated with increased risk of developing RA. In the first 2 years of RA (early RA), serum levels of IgM, IgG and IgA RF do not correlate with disease activity. Serum IgG and IgA RF in these years are prognostic of erosive joint disease. In established RA, high titer serum IgM RF correlates with the presence of articular disease and nodules but not with systemic disease activity. The presence of either IgG or IgA RF in patients with long-standing RA may be a good prognostic indicator of systemic manifestations. IgG and IgM RF are associated with extra-articular RA including rheumatoid vasculitis and nodules. The presence of IgM RF containing immune complexes with bound complement (C1q) is also associated with extra-articular RA.
**Principle of the Assay**

Fc fragments of highly purified human Immunoglobulin G are bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG, anti-human IgM and anti-human IgA immunologically detect the bound patient 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 IgG, IgM and IgA antibodies present in the original sample.
### General Information

#### Materials Supplied

<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-E (0; 15; 50; 150, 500 U/ml), containing serum/ buffer matrix (PBS, BSA, detergent, NaN₃ 0.09%), yellow. Ready to use.</td>
<td>5 vials, 1.5 ml each</td>
</tr>
<tr>
<td>Control positive (1) and negative (2), containing rheumatoid factor 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>2 vials, 1.5 ml each</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, IgA and IgM 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 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 deionized 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**
  - All reagents of this kit are intended for professional in vitro diagnostic 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 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.
  - Calibrators, Controls, 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 P
  Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a volume of 100 ml.

• Preparation of samples
  Dilute patient 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.

Sample Preparation

• 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 antibody activity.
• Testing of heat-inactivated sera is not recommended

Assay Procedure

Prepare enough microplate modules for all calibrators/ controls and patient samples.
1. Pipette 100 µl of calibrators, controls and prediluted patient 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 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 patient 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. Calibration is related to 1st British Standard Preparation 64/2 for Rheumatoid Factor.

- Measuring range
  The calculation range of this ELISA assay is 0-500 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 25 U/ml.

- Interpretation of results
  Negative: < 25 U/ml
  Positive: ≥ 25 U/ml

- Linearity
  Patient 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.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observe [U/ml]</th>
<th>Expected [U/ml]</th>
<th>O/E [%]</th>
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<tbody>
<tr>
<td>1</td>
<td>1:100</td>
<td>496.7</td>
<td>453.2</td>
<td>110</td>
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<tr>
<td>-</td>
<td>1:200</td>
<td>136.2</td>
<td>136.0</td>
<td>100</td>
</tr>
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<td>-</td>
<td>1:400</td>
<td>66.4</td>
<td>68.0</td>
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<tr>
<td>-</td>
<td>1:800</td>
<td>35.1</td>
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<tr>
<td>-</td>
<td>1:1600</td>
<td>16.8</td>
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<tr>
<td>-</td>
<td>1:3200</td>
<td>7.7</td>
<td>8.5</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
<td>1:500</td>
<td>262.4</td>
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<td>98</td>
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</table>

- **Limit of detection**
  Functional sensitivity was determined to be: 1 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.
  Intra-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 precision-within-assay are shown in the table below.
Intra-Assay Inter-Assay

<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>
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<td>321.0</td>
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- **Study results**

<table>
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<th>Study population</th>
<th>n</th>
<th>n Pos</th>
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<tr>
<td>Rheumatoid Arthritis</td>
<td>300</td>
<td>288</td>
<td>96.0</td>
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<tr>
<td>Normal human sera</td>
<td>169</td>
<td>19</td>
<td>11.2</td>
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Clinical Diagnosis

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<th>Pos</th>
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<tr>
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<td>19</td>
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<tr>
<td>Neg</td>
<td>12</td>
<td>159</td>
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</tbody>
</table>

| 300  | 169 | 469 |

Sensitivity: 96.0%
Specificity: 88.8%
Overall agreement: 93.4%
Resources

References


### Plate Layout

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<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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