Human Rheumatoid Factor IgG ELISA Kit

Enzyme Immunoassay for the quantification of Rheumatoid Factor IgG in serum or plasma.

Catalog number: ARG80410

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

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 an-tiglobulin 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, hyperglobulinemias, 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

This assay employs the quantitative enzyme immunoassay technique. Fc fragment of highly purified Immunoglobulin G has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated human antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C
Standards (0-4)	5 vials (0, 15, 50, 150, 500 U/ml)(Ready-to-use)	4°C
Control 1 (100 U/ml; acc. range: 75-125 U/ml)	1.5 ml (Ready-to-use)	4°C
Control 2 (2 U/ml; acc. range: < 10 U/ml)	1.5 ml (Ready-to-use)	4°C
5X Sample buffer	20ml	4°C
HRP-Antibody conjugate (IgG)	15ml (Ready-to-use)	4°C
50X Wash buffer	20ml	4°C
TMB substrate	15ml	4°C (Protect from light)
STOP solution	15ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.

- Store the kit at 4°C at all times.

- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.

- If crystals are observed in the 50X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.

- Ensure complete reconstitution and dilution of reagents prior to use.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.

- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

<u>Serum</u>- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

<u>Plasma</u> - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer.

- **1X Sample buffer:** Dilute 5X Sample buffer with distilled water before use.

- **Patient sample**: Dilute patient sample 1:100 with 1X sample buffer before assay, mix well. (e.g. 10 µl of sample + 990 µl of 1X sample buffer)

  Note: the controls and calibrators are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.

2. Add **100µl** of **standards**, **controls**, **samples** and **zero controls** into wells.

3. Incubate for **30 minutes** at **RT**.

4. Aspirate each well and wash, repeating the process 2 times for a <u>total 3 washes</u>. Wash by filling each well with 1× Wash Buffer (350µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

5. Add **100µl 1X HRP-antibody conjugate** into each well. Incubate for **15 minutes** at **RT**.

6. <u>Wash</u> as according to <u>step 4</u>.

7. Add **100µl** of **TMB Reagent** to each well. Incubate for **15 minutes** at **room temperature**.

8. Add **100µl of Stop Solution** to each well. Incubate for **5 minutes** at **RT**. The color of the solution should change from blue to yellow.

9. <u>Read the OD</u> with a microplate reader at <u>450nm</u> immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

INTERPRETATION OF RESULTS

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

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Rheumatoid IgG ranged from 3-500 U/ml. The mean MDD was 1 U/ml.

Interference

No interference has been observed with the following factors:

Haemolytic sera/plasma (up to 1000mg/dl)

Lipemic sera/plasma (up to 3g/dl triglycerides)

Bilirubin containing sera/plasma (up to 40mg/dl)

Antiboagulants (Citrate, EDTA, Heparin).

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6.6% and inter-assay precision was 7.1%.