Human Rheumatoid Factor IgM ELISA Kit

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

Catalog number: ARG80411

Package: 96 wells

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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, 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 RF titers 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 extraarticular RA.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. Fc fragment of highly purified human 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 anti-human IgM 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. Unused strips should be sealed tightly in the air-tight pouch.
Standard 0 - 4 (0, 15, 50, 150, 500 IU/ml)	5 X 1.5 ml (Ready-to-use)	4°C
Control 1 (100 IU/ml; acc. range: 75-125 IU/ml)	1.5 ml (Ready-to-use)	4°C
Control 2 (0 IU/ml; acc. range: < 10 IU/ml)	1.5 ml (Ready-to-use)	4°C
5X Sample buffer	20 ml	4°C
HRP-conjugated anti-human IgM Antibody	15 ml (Ready-to-use)	4°C
50X Wash buffer	20 ml (Ready-to-use)	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm (optional: reference filter at 620 nm (600-690nm))
- 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 2-8°C in the dark at all times. Do not expose reagents to heat, sun, or strong light during storage and usage.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- All materials must be at room temperature (20-28°C) prior to use.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use. Once started, perform the test without interruption.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- All incubation steps must be accurately timed.
- 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.

Serum- 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 for up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using Citrate EDTA or heparin 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 for up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
b) Samples containing sodium azide should not be used in the assay.
c) Testing of heat-inactivated sera is not recommended.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 20 ml of 50X Wash buffer + 980 ml of distilled water) The diluted Wash buffer is stable for 30 days at 2°C to 8°C.
- 1X Sample buffer: Dilute 5X Sample buffer with distilled water before use. (E.g. 20 ml of 5X Sample buffer + 80 ml of distilled water) The diluted Sample buffer is stable for 30 days at 2°C to 8°C.
- 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, 20-28 °C) 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 **100 µl** of **standards, controls and prediluted samples** into wells.
3. Incubate for **30 minutes at RT (20-28 °C)**.
4. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1× Wash Buffer (300µl)** 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 **100 µl** of **1X HRP-conjugated anti-human IgM Antibody** into each well. Incubate for **15 minutes at RT.**
6. **Wash** as according to step 4.
7. Add **100 µl** of **TMB Reagent** to each well. Incubate for **15 minutes at room temperature in dark**.
8. Add **100 µl** of **Stop Solution** to each well. **Incubate for 5 minutes at RT**. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at **450 nm** immediately (optional: Read the OD at 600-690nm as reference). It is recommended read the absorbance within 30 minutes after adding the stop solution.

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.

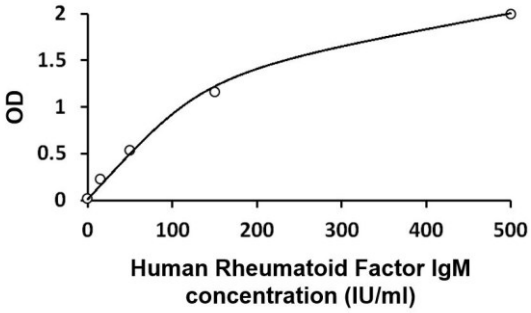
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If these quality control criteria (control 1: 75-125 IU/ml and control 2: <10 IU/ml; The OD (450/620nm) of the highest standard (500 IU/ml) should > 1.3; The OD (450/620nm) of the standard 0 (0 IU/ml) should < 0.15) are not met the assay run is invalid and should be repeated.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



The OD (450/620nm) of the highest standard (500 IU/ml) should > 1.3.

The OD (450/620nm) of the standard 0 (0 IU/ml) should < 0.15.

INTERPRETATION OF RESULTS

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

Negative: < 20 IU/ml

Positive: ≥ 20 IU/ml

QUALITY ASSURANCE

Sensitivity

The calculation range of this ELISA assay is from 0-500 IU/ml. The functional sensitivity was determined to be: 1 IU/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 4.4% and inter-assay precision was 7.4%.