



Instruction Manual

Rheumatoid Factor IgM ELISA

Enzyme immunoassay based on microtiter plate
for the detection and quantitative determination
of **Rheumatoid Factor (RF) IgM**
in serum and plasma



Cat. No.: ILE-RHF03
Storage: 2-8°C
For in-vitro diagnostic use only

October 2007

Contents	Page
1. Intended Use	3
2. General Information	3
3. Principle of the Test	3
4. Limitations, Precautions and General Comments	4
5. Reagents Provided	4
6. Materials Required but not Provided	5
7. Specimen Collection and Handling	6
8. Assay Procedure	6
9. Evaluation	7
10. Assay Characteristics	8
11. References	8

1. Intended Use

The IMMUNOLAB Rheumatoid Factor (RF) IgM ELISA Test Kit has been designed for the detection and the quantitative determination of RF in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of IMMUNOLAB.

This assay is intended for in-vitro diagnostic use only.

Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

2. General Information

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology. Rheumatoid arthritis is a systemic disease characterized by chronic proliferation and inflammation of joint cartilage and supporting structures. RA is mainly defined by clinical criteria, in which systematic pathogenetic studies have been hampered by doubts about the presence of common pathogenetic mechanisms and the relative lack of unique laboratory findings. IgG rheumatoid factor has been reported to be present in sera of patients with rheumatoid arthritis both with and without IgM rheumatoid factor activity. Rheumatoid factors are IgA, IgG and IgM immunoglobulins with antibody activity directed against antigenic sites on the Fc portion of IgG molecules. Because of its pentavalent structure and ability to cross-link immunoglobulin G antigen, IgM Rheumatoid Factor is the main class identified by clinically available diagnostic assays for Rheumatoid Factor detection. Rheumatoid factors may exist as the mu, gamma, alpha, and epsilon isotypes.

Rheumatoid factors are found in 1 to 4 % of the general population. They are present in 75% of adult patients with the highest incidence of rheumatoid factors occurring in persons over 65 years of age and nearly all patients with Felty and Sjogren syndrome. The clinical correlation of an elevated rheumatoid factor should be interpreted cautiously. Increased titers may accompany a variety of acute immune responses, particularly viral infections and a number of other diseases (e.g., infectious mononucleosis, tuberculosis, leprosy, various parasitic diseases, liver disease, sarcoidosis, and lymphoproliferative syndromes). The earliest tests and those still most widely used rely on the agglutinating properties of the IgM class of rheumatoid factors. Sensitized sheep red blood cell (Waalser-Rose) and latex agglutination tests have been developed and routinely employed. These assays are most sensitive for the detection of Rheumatoid factor that is of the IgM isotype because of its multivalent structure. These tests provide a dilution which is difficult to standardize and have laborious processing and poor reproducibility. In contrast to these assays modern ELISA tests are characterized by a higher sensitivity and by the possibility to differentiate between IgA, IgG and IgM Rheumatoid Factors.

3. Principle of the Test

The IMMUNOLAB RF IgM test kit is based on the principle of the enzyme immunoassay (EIA). Goat IgG is bound on the surface of the microtiter strips. Diluted patient serum, ready-to-use standards and controls are pipetted into the wells of the microtiter plate. A binding between the RF IgM of the serum and the immobilized goat IgG takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgM peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the RF IgM is directly proportional to the intensity of the color.

4. Limitations, Precautions and General Comments

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- No reagents from different kit lots have to be used, they should not be mixed among one another.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

5. Reagents Provided

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

Components	Volume / Qty.
Goat IgG coated microtiter strips	12
Standards with 0, 50, 200 and 500 IU/mL	4 x 2 mL
Positive Control	2 mL
Negative Control	2 mL
Enzyme Conjugate	15 mL
Substrate	15 mL
Stop Solution	15 mL
Sample Diluent	60 mL
Washing Buffer (10×)	60 mL
Plastic foils	2
Plastic bag	1

5.1. Mikrotiter Strips

12 strips with 8 breakable wells each, coated with affinity-purified goat IgG. Ready-to-use.

5.2. Standards

4 x 2 mL, human serum diluted with PBS, with 0, 50, 200 and 500 IU/mL of IgM Rheumatoid Factor. Addition of 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane. Ready-to-use.

5.3. Positive Control

2 mL, human serum diluted with PBS, contains IgM Rheumatoid Factor. The concentration range is given on the vial label. Addition of 0.02% methylisothiazolone and 0.02% bromonitrodioxane. Ready-to-use.

5.4. Negative Control

2 mL, human serum diluted with PBS, contains no IgM Rheumatoid Factor. Addition of 0.02% methylisothiazolone and 0.02% bromonitrodioxane. Ready-to-use.

5.5. Enzyme Conjugate

15 mL, anti-human-IgM-HRP (rabbit), in protein-containing buffer solution. Ready-to-use.

5.6. Substrate

15 mL, TMB (tetramethylbenzidine). Ready-to-use.

5.7. Stop Solution

15 mL, 0.5 M sulfuric acid. Ready-to-use.

5.8. Sample Diluent

60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

5.9. Washing Buffer

60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

5.10. Plastic Foils

2 pieces to cover the microtiter strips during the incubation.

5.11. Plastic Bag

Resealable, for the dry storage of non-used strips.

6. Materials Required but not Provided

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Bidistilled water

7. Specimen Collection and Handling

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

8. Assay Procedure

8.1. Preparation of Reagents

Washing Solution: dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- Standards and samples should be assayed in duplicates.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

8.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9. Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

9. Evaluation

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

Example

	OD Value	corrected OD	Mean OD Value
Substrate Blank	0.010		
Standard 1 (0 IU/mL)	0.039 / 0.041	0.029 / 0.031	0.030
Standard 2 (50 IU/mL)	0.744 / 0.698	0.734 / 0.688	0.711
Standard 3 (200 IU/mL)	1.318 / 1.360	1.298 / 1.350	1.324
Standard 4 (500 IU/mL)	2.035 / 2.123	2.025 / 2.113	2.069

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

9.1. Qualitative Evaluation

The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the 50 IU/mL standard. If the value of the sample is higher, there is a positive result.

For a value below the 50 IU/mL standard, there is a negative result. It seems reasonable to define a range of +/-20 % around the value of 50 IU/mL standard as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run.

9.2. Quantitative Evaluation

The ready-to-use standards and controls of the RF IgM kit are defined and expressed in International Units (IU/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for controls and standards in units are printed on the labels of the vials.

For a quantitative evaluation the absorptions of the standards and controls are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

10. Assay Characteristics

RF ELISA	IgG	IgA	IgM
Intra-Assay-Precision	6.3 %	6.2 %	4.4 %
Inter-Assay-Precision	2.4 %	9.1 %	7.3 %
Inter-Lot-Precision	1.0 – 3.2 %	6.5 – 9.3 %	2.6 – 13.8 %
Analytical Sensitivity	0.67 IU/mL	0.28 IU/mL	0.16 IU/mL
Recovery	95 – 119 %	80 – 113 %	71 – 113 %
Linearity	81 – 128 %	72 – 98 %	85 – 125 %
Cross-Reactivity	No cross-reactivity between Rheumatoid factors IgG, IgA and IgM.		
Interferences	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL		
Clinical Specificity	100 %	94 %	100 %
Clinical Sensitivity	100 %	100 %	91 %

11. References

1. Adebajo AO; Wright JK; Cawston TE; Hazleman BL: Rheumatoid factor quantitation: a comparison of ELISA and nephelometric methods. *Med Lab Sci* 1991 Jan; 48(1):47-51.
2. Banchuin N; Janyapoon K; Sarntivijai S; Parivisutt L: Re-evaluation of ELISA and latex agglutination test for rheumatoid factor detection in the diagnosis of rheumatoid arthritis. *Asian Pac J Allergy Immunol* 1992 Jun; 10(1):47-54.
3. Barka NE; Agopian MS; Peter JB: False-positive IgM antibodies to *Borrelia burgdorferi* in indirect ELISA as a result of IgM rheumatoid factor. *J Infect Dis* 1990 Jun; 161(6):1312.
4. Dabadghao S; Misra R; Naveed M; Aggarwal A: IgM rheumatoid factor estimation by ELISA in seronegative rheumatoid arthritis. *Rheumatol Int* 1996; 15(5):189-93.
5. Espersen GT; Ernst E; Vestergaard M; Grunnet N: ELISA estimations of rheumatoid factor IgM, IgA, and IgG in sera from RA patients with high disease activity. *Scand J Rheumatol Suppl* 1988; 75:40-5.
6. Gargiulo AV Jr; Toto PD; Robinson JA; Gargiulo AW: Latex slide agglutination vs. ELISA system. Rheumatoid factor detection. *J Periodontal Res* 1985 Jan; 20(1):31-4.
7. Gioud-Paquet M; Auvinet M; Raffin T; Girard P; Bouvier M; Lejeune E; Monier JC: IgM rheumatoid factor (RF), IgA RF, IgE RF, and IgG RF detected by ELISA in rheumatoid arthritis. *Ann Rheum Dis* 1987 Jan; 46(1):65-71.
8. Hoier-Madsen M; Grunnet N; Wiik A: A Danish inter-laboratory study of IgM rheumatoid factor (RF) determined by enzyme-linked immunosorbent assay (ELISA). *Scand J Rheumatol Suppl* 1988; 75:50-3.
9. Jonsson T; Arnason JA; Valdimarsson H: Enzyme-linked immunosorbent assay (ELISA) screening test for detection of rheumatoid factor. *Rheumatol Int* 1986; 6(5):199-204.
10. Karsh J; Halbert SP; Anken M; Klima E; Steinberg AD: Anti-DNA, anti-deoxyribonucleoprotein and rheumatoid factor measured by ELISA in patients with systemic lupus erythematosus, Sjogren's syndrome and rheumatoid arthritis. *Int Arch Allergy Appl Immunol* 1982; 68(1):60-9.
11. Lucic N; Mahic Zikic A; Lipa I; Seremet M: Comparison of the immunoenzyme test (ELISA) with other methods in the detection of rheumatoid factor. *Reumatizam* 1989; 36(1-6):24-30.