RF IgG (Rheumatoid Factor) ELISA

Catalog Number:
RFG31-K01

Enzyme immunoassay for the determination of Rheumatoid factor IgG
in human serum or plasma

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v. 1.0

INTENDED USE

The Eagle Biosciences RF IgG ELISA Assay Kit is used for the quantitative determination of rheumatoid factor (RF) IgG in human serum or plasma.

Patients suffering from rheumatoid arthritis (RA) exhibit RF, autoantibodies recognizing the Fc part of IgG. RA or chronic polyarthritis has a yet unknown etiology and represents the most frequent rheumatic inflammatory disorder demonstrating a prevalence rate of up to 1%. One of the typical manifestations of RA is symmetric synovialitis of limb joints often accompanied by involvement of the cervical spinal column.

Beside clinical features one of the criteria of the American College of Rheumatology for the diagnosis of RA is the presence of RF (1). Up to 80 % of RA patients may demonstrate RF. RF can occur years prior to the onset of disease and RF positive apparently healthy individuals bear a 5 - 40 times higher risk to develop RA (2). However, patients suffering from other autoimmune, infectious or B-cell lymphoproliferative disorders as well as apparently healthy elderly individuals may develop RF.

High concentrations of RF are often associated with a more severe disease comprising a faster destruction of joints. In addition, they are found in patients with extra-articular manifestations such as rheumatoid nodules, polyneuropathy, vasculitis or Sicca syndrome.

RF may belong to the IgG, IgM or IgA isotype whereas IgM RF is the most frequent isotype to be determined in RA patients. Extra-articular manifestations seem to be associated with IgA RF. Like RF of the IgM isotype high concentrations of IgG RF seems to appear with patients suffering from more progressive erosions of joints.

Eagle Biosciences offers a complete range of serological markers for systemic autoimmune diseases. All assays employ the same assay scheme and predilution maximizing laboratory efficiency.

(1) Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988 31 315-24
(2) MacGregorFJ, Silman AJ.: Rheumatoid factors as predictors of rheumatoid arthritis. J Rheumatol 1991 18 1280-1

PRINCIPLE OF THE TEST

The Eagle Biosciences RF IgG ELISA Assay Kit is an enzyme immunoassay for the quantitative determination of IgG antibodies to the Fc region of IgG in human serum or plasma.

The rheumatoid factors of the calibrators, control and diluted patient samples react with rabbit IgG immobilized on the solid phase of microtiter plates. Following an incubation period of 60 min at room temperature, unbound sample components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP). Within the incubation period of 30 min at RT, excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibody concentrations of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not used.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at- 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: ***Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1.0 ml sample diluent (C), prior to assay.***

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

TEST COMPONENTS FOR 96 DETERMINATIONS

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells (total 96 individual wells) coated with rabbit IgG	1 vacuum sealed with desiccant, 2 adhesive foils
B BUF WASH	**Concentrated wash buffer** sufficient for 1000 ml solution 10x	100 ml concentrate capped white
C DIL	**Sample diluent**	100 ml ready for use capped black
D CONJ	**Conjugate** containing anti-human-IgG- (sheep) coupled with HRP	15 ml ready for use capped red
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F H2SO4	**Stop solution** 0.25 M sulphuric acid 0.25M	15 ml ready for use capped yellow
0 - 4 CAL	**Calibrators** (diluted serum) conc.: 1, 10, 30, 100, 300 IU/ml)	1 ml each ready for use capped white
P CONTROL	**Positive control** (diluted serum) conc.: see leaflet enclosed +	1 ml ready for use capped red
N CONTROL	**Negative control** (diluted serum) conc.: see leaflet enclosed -	1 ml ready for use capped green

Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water

Size and storage

The Eagle Biosciences RF IgG ELISA Assay Kit has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the RF IgG ELISA Assay Kit have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water.
For example, dilute 8 ml of the concentrate with 72 ml of distilled water.
The wash solution prepared is stable at 2 - 8 °C up to 30 days.

Crystallization of the undiluted washing buffer may occur and can be dissolved by warming up to 37 °C.

The soak time of the washing buffer should be 5 seconds per washing step.

<u>Avoid exposure of the TMB substrate solution to light!</u>

ASSAY PROCEDURE

- **Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C)**.
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18…25°C) before use. Mix gently without causing foam.
2. Dispense
   100 µl calibrators 1 - 4 (CAL 0 optionally)
   100 µl positive control (P)
   100 µl diluted patient samples
   into the respective wells.
3. Cover plate, incubate **60 min** at room temperature (18…25°C).
4. Decant, then wash each well **three** times using **300 µl**
   wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Cover plate, incubate **30 min** at room temperature (18…25°C).
7. Decant, then wash each well **three** times using **300 µl** of wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Cover plate, incubate **15 min protected from light** at room temperature (18…25°C).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within 30 min after adding the stop solution.

DATA PROCESSING

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 (CAL 0 optionally) on the ordinate, y-axis, (lin. scale) versus their respective RF IgG concentrations on the abscissa, x-axis, (log. scale).

RF IgG concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

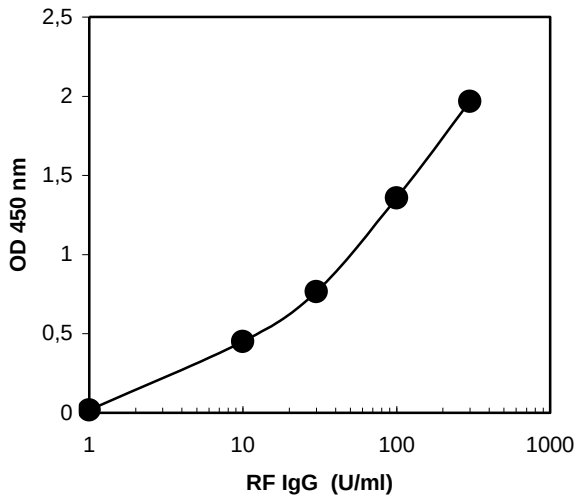
Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

RF IgG may be used also with Computer Assisted Analysis using software able to plot log/lin curves with four-parameter fit.

Example of Typical Assay Results

Well	OD (a)	OD (b)	OD (mean)	U/ml
Calibrator **0**	0.018	0.018	0.018	**1**
Calibrator **1**	0.443	0.459	0.451	**10**
Calibrator **2**	0.772	0.760	0.766	**30**
Calibrator **3**	1.368	1.347	1.358	**100**
Calibrator **4**	1.971	1.963	1.967	**300**
Patient **1**	1.109	1.098	1.104	**62.2**

TYPICAL STANDARD CURVE



Specimens with an OD > calibrator 4 should be diluted with RF negative serum and tested again. Results are to be multiplied with the dilution factor chosen.

Test validity

The test run is valid if:

- the mean OD of the calibrator 1 is ≤ 0.6
- the mean OD of the calibrator 4 is ≥ 1.2

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

RF IgG	U/ml
negative	**< 25**
grey zone	**25 - 30**
positive	**> 30**

Specimens with concentrations detected in the grey zone should be tested again.

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum RF IgG levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method

Healthy individuals should be tested negative by the RF IgG. However, RF positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA

Calibration

RF IgG is calibrated in arbitrary units U/ml.

Linearity

Dilutions of selected positive specimens in RF IgG autoantibody free human serum are determined according to their expected theoretical values with RF IgG.

Sensitivity

The analytical sensitivity of the RF IgG is 1.5 U/ml.

Normal distribution

137 normal sera from patients without clinical symptoms were tested in the RF IgG. All but one serum were found negative. This corresponds to a specificity of >99%.

Precision

Intraassay coefficient of variation (CV) in RF IgG from 8fold sample determination:

Sample	U/ml	CV (%)
Serum 1	181	2.29
Serum 2	105	5.38
Serum 3	52	3.48
Serum 4	18	5.53

Interassay coefficient of variation (CV) in RF IgG from 9 different determinations of sample triplicates:

Sample	U/ml	CV (%)
Serum A	178	8.38
Serum B	114	8.44
Serum C	84	7.62
Serum D	21	5.31

ASSAY SCHEME

Dilute patients sample 10 µl serum + 1.0 ml sample diluent (made of C)

1	Bring all ready for use reagents to room temperature (18…25°C) before use.			
		calibrators	control	sera
2	Pipette Calibrators (0 - 4)	100 µl		
	Positive Control (P)		100 µl	
	prediluted 1 + 100 patient sera			100 µl
3	Incubate	60 minutes at room temperature		
4	Wash	Decant, Dispense 3 x 300 µl (made of B)		
5	Pipette conjugate (D)	100 µl	100 µl	100 µl
6	Incubate	30 minutes at room temperature		
7	Wash	Decant, Dispense 3 x 300 µl (made of B)		
8	Pipette substrate (E)	100 µl	100 µl	100 µl
9	Incubate protected from light	15 minutes at room temperature		
10	Pipette stop solution (F)	100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm			

SAFETY PRECAUTIONS

- **This Eagle Biosciences RF IgG ELISA Assay Kit is for research use only**. Follow the working instructions carefully. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the RF IgG ELISA Assay Kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.