



AUTOZYME™ RF

Rheumatoid Factor IgA, IgM and IgG (Class Specific)

- REF** Z9196: Rf IgA kit
- REF** Z9296: Rf IgM kit
- REF** Z9396: Rf IgG kit

Instructions for Use

IVD For in vitro diagnostics use only

96 Tests

Store at 2 - 8°C



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Kit Contents Symbols

- CAL** Calibrators
- CONTROL -** Negative Control
- CONTROL +** Positive Control
- BUF WASH** Wash Buffer concentrate
- DIL SPE** Sample Diluent
- CONJ** Conjugate solution
- SUB** Substrate solution
- STOP** Stop Solution
- SORB** Solid Phase – Antigen Coated Wells

1. Intended Use

The Rheumatoid Factor IgA, IgM and IgG assays have been designed for the direct quantitation of rheumatoid factor IgA, IgM and IgG in human serum. The assays are calibrated using the WHO Reference Reagent Rheumatoid Arthritis Human Serum, W1066 which replaced the 1st British Standard 64/2. Reagents are for *in vitro* use only.

2. Background

The presence of rheumatoid factors (auto-antibodies directed against the Fc region of IgG molecules) is a common feature of rheumatoid arthritis. Rheumatoid factors have been found among the IgM, IgA and IgG classes of immunoglobulin. Most agglutination methods detect 19S (pentameric) IgM rheumatoid factor (Rf) only. Using ELISA technology, all major immunoglobulin classes can be measured.

The IgM Rf shows a strong correlation with the onset of an erosive disease state. However, IgM Rf is also present in patients with SLE and bacterial endocarditis. Recent literature suggests that 75% of patients with chronic polyarthritis have IgM Rf whereas only 30% of patients with other connective tissue diseases have raised levels. IgM Rf is also seen in other diseases such as viral hepatitis, liver cirrhosis, sarcoiditis and tuberculosis.

IgG Rf has been reported to be significantly increased in patients with rheumatoid vasculitis and correlate with disease activity. Moreover, IgG Rf may contribute to the tissue damage by activating complement.

The presence of IgA Rf is indicative of a more severe and erosive outcome of the disease. The detection of IgA Rf can give an early indication of an underlying rheumatic disease and is considered to be more specific than IgM Rf.

3. Principle

The AUTOZYME™ method employs antigen coated microwell technology, which is ideal for batch screening of large and small numbers of samples for rheumatoid factor IgA, IgM and IgG. The method employs a sandwich enzyme immunoassay (EIA) principle.

First incubation:

Purified antigen (horse IgG) is coated in the AUTOZYME™ Rf wells. Calibrators or diluted samples are pipetted into the wellstrips, allowing any antibodies present to bind to the well surface. The wells are then washed with wash buffer.

Second incubation:

Anti-IgA, IgM or IgG-peroxidase conjugate is then added to the wells. Any Rf IgA, IgM or IgG bound to the wells will bind conjugate. Unbound conjugate is removed by washing.

Third incubation:

A pale green substrate is then added to the wells. The intensity of the green colour formed is proportional to the concentration of Rf IgA, IgM or IgG bound in the first incubation. The reaction is stopped with a low pH solution.

4. Kit Contents

5 vials calibrators (5 Rf IgA or 5 Rf IgM or 5 Rf IgG), 1.5mL each (ready-to-use).

Calibrator	Rf IgA (ARF U/mL)	Rf IgM (MRF U/mL)	Rf IgG (GRF U/mL)
1	0.0	0.0	0.0
2	22.2	22.2	22.2
3	66.7	66.7	66.7
4	200.0	200.0	200.0
5	600.0	600.0	600.0

- 1 vial wash buffer concentrate (PBS/Tween), 100 mL
- 2 x vial sample diluent, yellow colour (BSA/PBS), 50 mL
- 1 vial conjugate: (anti-human IgA or IgG or IgM HRP), 15 mL
- 1 vial substrate (ABTS), 15 mL
- 1 vial stopping buffer (oxalic acid), 15 mL
- 1 foil sachet, containing 1 set of antigen-coated microwells
- 1 vial Positive control, 1.5 mL (ready-to-use)
- 1 vial Negative control, 1.5 mL (ready-to-use)
- 1 instruction leaflet
- 1 QC certificate

5. Storage

The kit should be stored refrigerated at 2-8°C. Do not use the reagents beyond their expiry date. Do not freeze. Keep all reagents away from direct sunlight.

6. Sample Handling

AUTOZYME™ Rf must be performed with human serum samples. Samples should be assayed within 24 hours of collection or stored frozen at -15°C or colder. Repeated freeze-thawing is not advisable. Do not heat treat samples prior to assay.

7. Additional Reagents and Equipment Required

- Deionised or freshly distilled water.
- Precision micropipettes to deliver 10 - 1000 µL.
- Multichannel micropipette or repeating dispenser to deliver 100 µL.
- 2000 mL measuring cylinders for reagent preparation.
- Automated plate washer (optional).
- 96-well microplate reader with 405 nm filter.
- Software package (optional).

8. Procedural Precautions

Numbering of each strip is advised prior to commencing the assay. Allow all reagents to equilibrate to room temperature (18°C to 25°C) before use for a minimum of 2 hours.

Avoid the use of icteric, lipaemic or grossly haemolysed samples.

Always change tips between different calibrators, samples or control sera to prevent sample carryover.

Never allow the same pipette tip to be used with different reagents. Special care is needed to prevent contamination of the substrate by the conjugate.

The substrate should be pale green. Any green colouration above 0.200 indicates substrate contamination and should be discarded.

The well washing procedure is critical for the successful performance of the test, especially between conjugate and substrate incubations (i.e. the second and third incubations).

AUTOZYME™ Rf has been designed so that all the AUTOZYME™ Rf assays (IgA, IgM and IgG) can be run simultaneously. The substrate, stop, wash buffer, wells and sample diluent are common components of AUTOZYME™ Rf assays and are not lot specific.

Do not interchange kit components from different lots.

Do not use the kit beyond the expiry date given on the label. Unused reagents are stable at 2 - 8°C for 1 month after first opening the container. However, multiple re-use could increase the risk of reagent contamination.

9. Assay Procedure

1. Prepare the wash buffer as follows: dilute contents of the **wash buffer concentrate** vial to 2000 mL with deionised water or proportionally less if not using the whole kit.
2. Dilute the patient samples by 1/100 using the sample diluent e.g. 10 µL sample added to 990 µL diluent.
The **Calibrators and kit Controls** do not require dilution.

- Remove the **antigen-coated microwells** from the foil sachet and seal any unrequired wells in the resealable **foil sachet**, along with the desiccant sachet.
- Dispense 100 μL of each **calibrator**, **kit control** or **diluted patient sample** into appropriate wells. Incubate for 30 minutes at room temperature (18 - 25°C). Samples and calibrators should be dispensed within 10 minutes of commencing the assay. It is recommended that samples be tested in duplicate.
- Gripping the frame on the long sides to retain the strips, flick out the contents of the wells. Using the diluted wash buffer, wash the wells at least three times either with an automated plate washer set to at least 300 μL per well, or by adding 300 μL to each well and flicking out, gripping the frame on the long sides to retain the strips. Alternatively use a wash bottle. Blot the wells on absorbent material to remove any residual liquid.
- Add 100 μL of **conjugate** to each well and incubate for 30 minutes at room temperature (18 - 25°C).
- Gripping the frame on the long sides to retain the strips, flick out the contents of the wells. Wash the wells at least three times using the same procedure as in step 5.
- Dispense 100 μL of **substrate** into each well, ensuring that it is initially pale green and incubate for 30 minutes at room temperature (18 - 25°C).
- Stop the reaction by adding 100 μL of stopping buffer.
- Measure the absorbance at 405 nm on a 96-well microplate reader.

10. Calculation of Results

For each assay, prepare a calibration curve by plotting mean absorbance against calibrator concentration on linear graph paper, and interpolate unknowns. Alternatively, use a computerised curve-fit program.

Any sample giving values above the calibrator range should be diluted and retested.

11. Quality Control

Quality control samples for the AUTOZYME™ Rf assays are provided within the kits. Good laboratory practice requires that quality control samples be included in every run to check the assay performance.

Target ranges for the controls are quoted on the QC certificate. If either control falls outside the quoted range, the results are invalid and the assay should be repeated.

12. Performance Characteristics

12a Minimum detectable concentration:

The minimum detectable concentration, defined as the concentration equal to 2 standard deviations from the mean of 20 replicate determinations of the sample diluent, was found to be:

rheumatoid factor IgA	2.9 ARF U/mL
rheumatoid factor IgM	1.1 MRF U/mL
rheumatoid factor IgG	1.7 GRF U/mL

13. Safety Precautions

For *in vitro* diagnostic use only.

For Professional Use only.

Safety data sheets are available on request.

The **substrate** contains ABTS™ which is harmful if swallowed in copious amounts and may cause skin irritation if exposed for prolonged periods. In case of skin contact, wash with soap and water. Flush eyes with copious amounts of water. The **calibrators and controls** contain human source material. Although found negative when tested for HIV-1 and HIV-2 antibodies, HCV and hepatitis B surface antigen, no test can guarantee their absence. Therefore, the calibrators should be handled using the same safety precautions employed when handling any potentially infectious material. In case of contact with any reagent, immediately flush eyes or skin with water. If ingested, wash out mouth with water and obtain medical attention immediately.

Used calibrators, controls, samples, pipette tips and plates should be handled as clinical waste and incinerated or disposed of in accordance with local rules. Other reagents should be diluted and flushed down the drain. It is recommended that gloves are worn when handling such items.

 **STOP** Stop Solution contains oxalic acid.

Signal Word: Warning

Hazard Statements: H302 - Harmful if swallowed
H312 - Harmful in contact with skin

Precautionary Statements:

P280 - Wear protective gloves/ eye protection/face protection.

P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes.

Remove contact lenses, if present and easy to do. Continue rinsing.

ABTS™ (2, 2'-azino-bis (3-ethylbenzothiazoline-6 sulphonic acid) is a trademark of Roche Diagnostics.

Bibliography

- Ernst, E *et al* (1988) RF-class (IgM, IgG, IgA) in a group of highly active RA-patients in relation to disease activity and treatment. *Scan. J. Rheum. Suppl.* **75**, 250-255.
- Gioud-Paquet, M. *et al* (1987) IgM rheumatoid factor (RF), IgA RF, IgM RF and IgG RF detected by ELISA in rheumatoid arthritis. *Ann. Rheum. Diseases*, **46**, 65-71.
- Müller, K. *et al* (1989) Circulating IgA- and IgM-rheumatoid factors in patients with primary Sjögren syndrome. *Scand. J. Rheum.* **18**, 29-31.
- Pope, R. M., Lessard, J. and Nunnery, E. (1986) Differential effects of therapeutic regimens on specific classes of rheumatoid factor. *Ann. Rheum. Diseases*, **45**, 183-189.
- Teitsson, I. (1988) IgA rheumatoid factor as predictor of disease activity. *Scan. J. Rheum. Suppl.* **75**, 233-237.
- Westedt, M. L. *et al* (1985) Rheumatoid factors in rheumatoid arthritis and vasculitis. *Rheumatol. Int.* **5**, 209-237.
- Winska Wilooh, H. *et al* (1988) IgA and IgM rheumatoid factors as markers of later erosive changes in rheumatoid arthritis (RA). *Scan. J. Rheum. Suppl.* **75**, 238-243.
- Withington, R. H. *et al* (1984) Felty's syndrome associated with high levels of IgA rheumatoid factor. *Ann. Rheum. Diseases*, **43**, 505-507.
- Withington, R. H. *et al* (1984) Prospective study of early rheumatoid arthritis. II. Association of rheumatoid factor isotypes with fluctuations in disease activity. *Ann. Rheum. Diseases*, **43**, 679-685.
- Rudge, S. R. *et al* (1985) Class specific rheumatoid factors in rheumatoid arthritis: response to chryotherapy and relationship to disease activity. *J. Rheumatol.* **12**, 432-436.
- Moore, T. L. *et al* (1988) Enzyme linked (ELISA) immunosorbent assay for the detection of hidden 19S IgM rheumatoid factors in juvenile rheumatoid arthritis. *J. Rheumatol.* **15**, 87-90.
- Adebajo, A. O. *et al* (1991) Rheumatoid factor quantitation: a comparison of ELISA and nephelometric methods. *Medical Laboratory Sciences*, **48**, 47-51.
- Quismorio, F. P. *et al* (1983) IgG rheumatoid factors and anti-nuclear antibodies in rheumatoid vasculitis. *Clin. exp. Immunol.* **52**, 333-340.

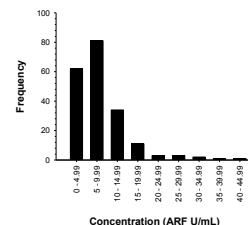
12b Precision data:

Intra-assay (n=20)	ARF U/mL	CV %	MRF U/mL	CV %	GRF U/mL	CV %
Sample 1	10.3	5.0	14.2	3.3	9.0	3.2
Sample 2	50.7	2.3	41.3	4.6	36.5	2.3
Sample 3	104.9	4.0	281.2	4.3	164.9	3.2
Inter-assay (n=10)	ARF U/mL	CV %	MRF U/mL	CV %	GRF U/mL	CV %
Sample 1	6.1	16.0	3.8	16.7	16.9	14.4
Sample 2	184.0	11.1	195.2	11.0	102.3	11.6
Sample 3	330.6	11.1	361.6	12.3	287.7	15.8

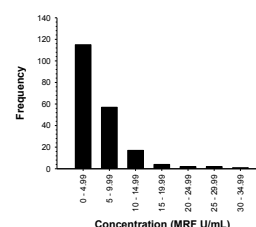
12c Reference values:

AUTOZYME™ Rf was used to determine the Rf IgA, IgM and IgG levels of 100 serum samples (tested in duplicate) from normal blood donors with no apparent abnormalities. The data was evaluated and the following ranges obtained:

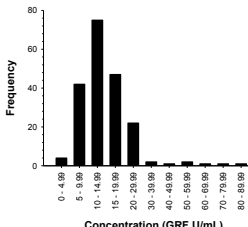
Normal range ≤ 21.4 ARF U/mL
Positive > 21.4



Normal range ≤ 15.3 MRF U/mL
Positive > 15.3



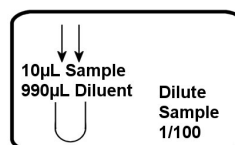
Normal range ≤ 35.9 GRF U/mL
Positive > 35.9



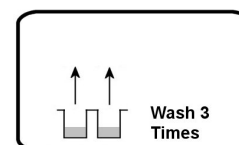
It is recommended that each laboratory determines its own reference range.

Test Procedure

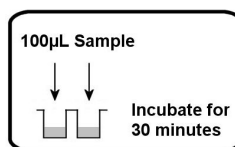
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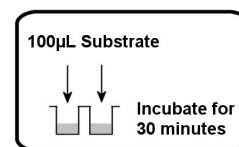
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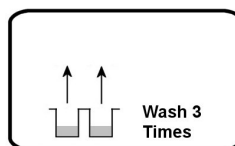
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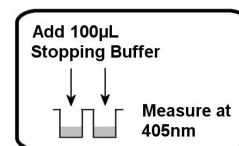
6.



3.



7.



4.

