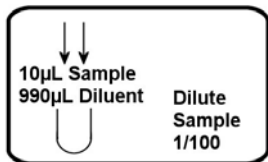
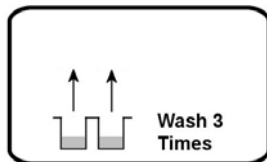


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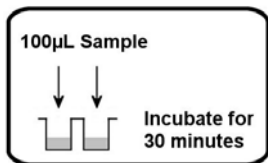
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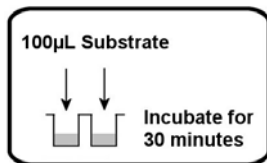
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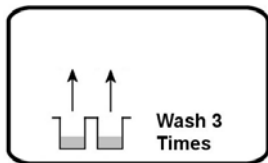
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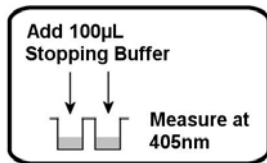
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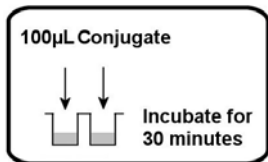
3.



7.



4.



AUTOZYME™ RF

Rheumatoid Factor
IgA, IgM and IgG (Class Specific)

IgA: Z9196

IgM: Z9296

IgG: Z9396

Instructions for Use

A5297.12
Apr '05



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Table of Contents

Intended Use	3
Background	3
Principle	3
Kit Contents	4
Storage	5
Sample Handling	5
Additional Reagents and Equipment Required	5
Procedural Precautions	5
Assay Procedure	6
Calculation of Results	7
Quality Control	7
Performance Characteristics	8
Safety Precautions	11
Bibliography	12

Kit Contents Symbols

CAL	Calibrators
CONTROL -	Negative Control
CONTROL +	Positive Control
BUF WASH	Wash Buffer
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 British Reference Preparation (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.

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13. Safety Precautions

For *in vitro* diagnostic use only.
For Professional Use only.

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.

Safety data sheets are available on request.

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

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), 33 mL

1 vial sample diluent, yellow colour (BSA/PBS), 100 mL

1 vial conjugate:

(anti-human-IgA or IgM or IgG-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 to 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.

1000 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.

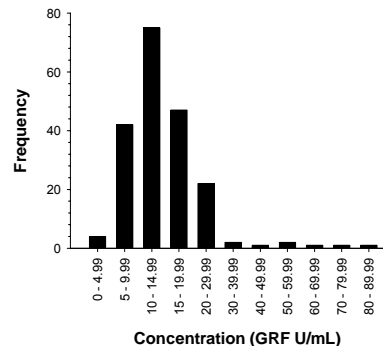
Allow all reagents to equilibrate to room temperature (18°C to 25°C) before use for a minimum of 2 hours.

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

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

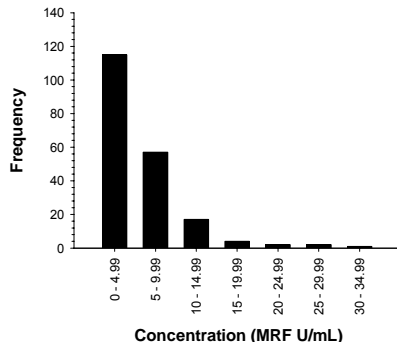
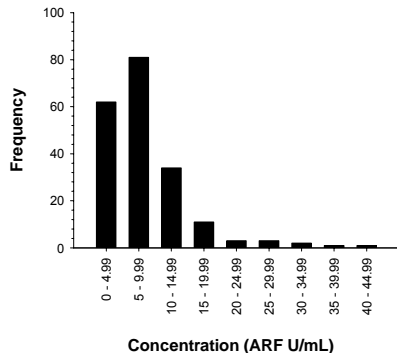
Never allow the same pipette tip to be used with different reagents. Special care is needed to prevent contamination of the substrate by the conjugate.

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



It is advisable that each laboratory establishes its own reference range.

	Rf IgA ARF U/mL	Rf IgM MRF U/mL	Rf IgG GRF U/mL
Normal range	≤ 21.4	≤ 15.3	≤ 35.9
Positive	> 21.4	> 15.3	> 35.9



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 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 1000 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.
3. Remove the **antigen-coated microwells** from the foil sachet and seal any unrequired wells in the resealable **foil sachet**, along with the desiccant sachet.
4. 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.
5. 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.
6. Add 100 µL of **conjugate** to each well and incubate for 30 minutes at room temperature (18 - 25°C).

7. 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.
8. Dispense 100 μ L of **substrate** into each well, ensuring that it is initially pale green and incubate for 30 minutes at room temperature (18 - 25°C).
9. Stop the reaction by adding 100 μ L of stopping buffer.
10. 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

1. Precision data:

Intra-assay (n=20)	ARF U/mL	CV %	MRF U/mL	CV %	GRF U/mL	CV %
Sample 1	4.8	23.5	3.0	6.9	16.5	2.9
Sample 2	204.6	5.2	196.2	9.5	116.1	8.4
Sample 3	345.7	5.7	363.3	13.1	320.1	6.8
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

2. Minimum detectable concentration:

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

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

3. 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: