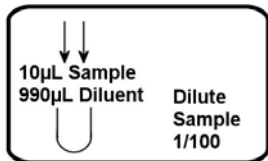
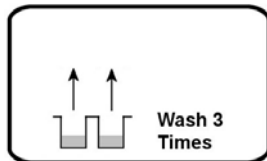


Test Procedure

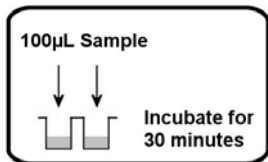
1.



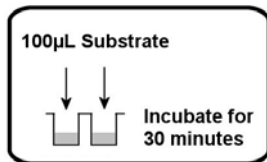
5.



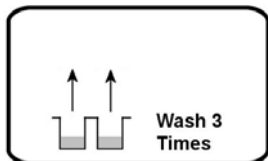
2.



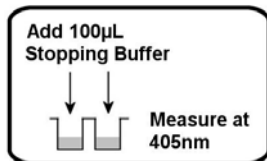
6.



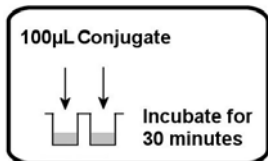
3.



7.



4.



AUTOZYME™ RF Screen

Rheumatoid Factor Screen (All Classes)

Z9096

Instructions for Use

A5298.04
May '05



Cambridge Life Sciences Ltd
14 St. Thomas' Place
Cambridgeshire Business Park
Ely, Cambs. CB7 4EX UK
Tel: +44(0)1353 645200 Fax: +44(0)1353 645250
email: sales@clsdiagnostics.com
www.clsdiagnostics.com

A small icon of a factory with three chimneys is located above the contact information.

Table of Contents

Intended Use	3
Background	3
Principle	4
Kit Contents	4
Storage	5
Sample Handling	5
Additional Reagents and Equipment Required	5
Procedural Precautions	6
Assay Procedure	7
Calculation of Results	8
Quality Control	8
Performance Characteristics	9
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 AUTOZYME™ RF Screen assay has been designed for the quantitative detection of rheumatoid factor of all classes in human serum. The assay is calibrated against the British Reference Preparation (64/2).

2. Background

Rheumatoid factor (RF) is found in the serum of the majority of patients affected by rheumatoid arthritis and appears in non-rheumatoid diseases such as systemic lupus erythematosus, sub-acute bacterial endocarditis and leprosy. It can also be detected at low levels in the serum of some normal individuals.

Rheumatoid factor was discovered over 40 years ago. Since then it has been identified as an immunoglobulin against class G immunoglobulins (IgG). RF refers to a heterogeneous group of antibodies, both in immunoglobulin class and specificity.

Rheumatoid factor plays a major role in the pathogenesis of rheumatoid arthritis through the deposition of immune complexes in the tissues, particularly the synovial spaces between the joints, leading to a painful swelling in affected patients. The presence of IgG RF particularly indicates the presence of active disease processes.

The measurement of RF is an important diagnostic tool in the detection and long-term management of the disease in suspected rheumatoid patients.

Bibliography

1. 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. *Scand. J. Rheum*, Suppl. 75, 250 - 255.
2. 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.
3. Müller, K. *et al.* (1989) Circulating IgA- and IgM-rheumatoid factors in patients with primary Sjögren syndrome. *Scand. J. Rheum*, 18, 29 - 31.
4. 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.
5. Quismorio, F. P. *et al* (1983) IgG rheumatoid factors and anti-nuclear antibodies in rheumatoid vasculitis. *Clin. Exp.Immunol*, 52, 333 - 340.
6. Teitsson, I. (1988) IgA rheumatoid factor as predictor of disease activity. *Scand. J. Rheum*, Suppl. 75, 233-237.
7. Westedt, M. L. *et al* (1985) Rheumatoid factors in rheumatoid arthritis and vasculitis. *Rheumatol. Int.*, 5, 209 - 237.
8. Winska Wiloch, 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.
9. Withrington, R. H. *et al* (1984) Felty's syndrome associated with high levels of IgA rheumatoid factor. *Ann. Rheum. Diseases*, 43, 505 - 507.
10. Withrington, 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.

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 **kit controls** contain human source material. Although found negative when tested for HCV, HIV antibodies and hepatitis B surface antigen, no test can guarantee their absence. Therefore, the calibrators and kit controls 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.

3. Principle

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

First incubation:

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

Second incubation:

Conjugate is then added to the wells. Any RF 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 bound in the first incubation. The reaction is stopped with a low pH solution.

4. Kit Contents

6 vials calibrators, (1.5mL each ready-to-use).

Calibrator	Rf Screen (IU/mL)
1	0.0
2	12.5
3	25.0
4	50.0
5	100.0
6	200.0

- 1 vial wash buffer concentrate, 33 mL
- 1 vial sample diluent yellow colour, 100 mL
- 1 vial conjugate, (anti-human IgA/IgM/IgG - HRP) 15 mL
- 1 vial substrate (ABTS), 15 mL
- 1 vial stop solution (oxalic acid), 15 mL
- 1 foil sachet containing antigen-coated AUTOZYME™ wells
- 1 vial Negative Control, 1.5 mL (ready-to-use)
- 1 vial Positive Control, 1.5 mL (ready-to-use)
- 1 instruction leaflet
- 1 QC certificate

5. Storage

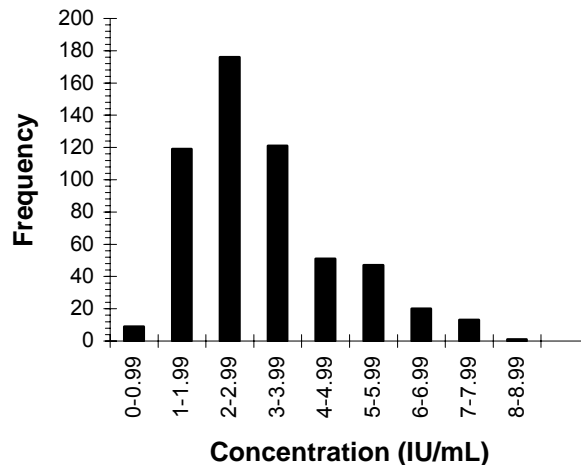
The kits should be stored refrigerated at 2 - 8°C. Do not use the reagents beyond their expiry date. Do not freeze. Keep all reagents away from direct sunlight.

6. Sample Handling

AUTOZYME™ RF screen may be performed on 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.

7. Additional Reagents and Equipment Required

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



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

12. Performance Characteristics

a. Precision data:

Intra-assay (n=20)	Mean IU/mL	CV %
Sample 1	1.9	9.8
Sample 2	76.5	4.0
Sample 3	112.4	3.6

Inter-assay (n=8)	Mean IU/mL	CV %
Sample 1	2.3	13.9
Sample 2	75.8	4.9
Sample 3	114.2	4.1

b. 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 0.53 IU/mL.

c. Reference values:

AUTOZYME™ RF screen was used to determine the RF levels of 100 serum samples measured in duplicate from normal blood donors with no apparent abnormalities, in three reagent batches. The data was evaluated and the following range obtained:

	IU/mL
Normal range	≤8.0
Positive	>8.0

8. Procedural Precautions

Numbering of each strip is advised prior to commencing the assay.

Allow all reagents to equilibrate to room temperature (18 - 25°C) before use for a minimum of two 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 absorbance indicates substrate contamination and the substrate 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 (Z9096/Z9196/Z9296/Z9396) can be run simultaneously. The wash buffer, wells, sample diluent, substrate and stop are common.

Do not use the kit beyond the expiry date given on the label.

Unused reagents are stable at 2 - 8°C for up to 28 days 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 vial **wash buffer concentrate** to 1000mL with deionised water.
2. Dilute the patient samples 1/100 e.g. 10 µL sample + 990µL 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 **foil sachet**, along with the desiccant sachet.
4. Dispense 100µL 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. Flick out the contents of the wells over a sink. Wash the wells three times by adding 300µL of wash buffer to each well with either an automated washer or manually (alternatively use a wash bottle). Flick out the wash buffer over a sink. Repeat two more times then blot the wells on absorbent material to remove any residual liquid.
6. Add 100µL of **conjugate** to each well and incubate for 30 minutes at room temperature (18 - 25°C).
7. Flick out the contents of the wells over a sink. Wash the wells three times using the same procedure as in step 5.
8. Dispense 100µL 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 100µL of **stopping buffer**.

10. Measure the absorbance of each well at 405nm 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 re-tested.

11. Quality Control

Good laboratory practice requires that quality control samples be included in every run to check the assay performance.

The kit control ranges are provided on the QC certificate.

If either of the control values falls outside the quoted range, the results are invalid and the assay should be repeated.