

AUTOZYME™ ENA

(Enzyme immunoassay for the qualitative detection of auto-antibodies to extractable nuclear antigens)

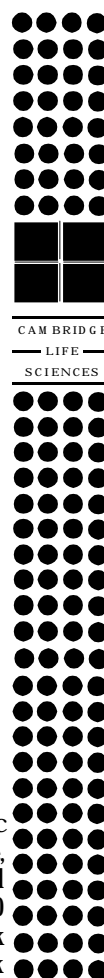
Codes:

Bulk pack:	Z1000
Well packs:	
Anti-Ro:	Z1100
Anti-La:	Z1200
Anti-Sm:	Z1300
Anti-Sm/RNP:	Z1400
Anti-Jo-1:	Z1500
Anti-Scl-70:	Z1600

Instructions for Use

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Cambridge Life Sciences plc
Cambridgeshire Business Park, Angel Drove,
Ely, Cambs. CB7 4DT. England
Tel. 01353 645200 Fax 01353 645250
email: clssales@byk.co.uk
www.cambridgelifesciences.co.uk



1. Intended Use

AUTOZYME™ ENA is a panel of sandwich enzyme immunoassays (EIA) for the qualitative detection of Ro, La, Sm, Sm/RNP, Jo-1 and Scl-70 auto-antibodies in human serum or plasma.

2. Background

The AUTOZYME™ ENA range of assays offers significant technical improvements over existing methods, for example countercurrent immunoelectrophoresis and Ouchterlony diffusion, in terms of speed, flexibility, differential diagnosis and sensitivity, for the detection of antibodies to extractable nuclear antigens (ENAs).

The system allows the flexibility for any combinations of these analytes to be run simultaneously, using an identical procedure.

The clinical indications for these antibodies are:

- anti-Ro (SS-A) - Sjögren's syndrome (70-80%)
- systemic lupus erythematosus (SLE) (30-40%)
- anti-La (SS-B) - Sjögren's syndrome (60-70%)
- SLE (10-15%)
- anti-Sm - SLE (8-40%)
- anti-Sm/RNP - mixed connective tissue disease (95-100%)
- lower frequency in SLE
- anti-Jo-1 - polymyositis (25-35%)
- anti-Scl-70 - systemic sclerosis (CREST) (70-90%)

Reactivities have been characterised by reference to the following control panels:

Centre for Disease Controls, Atlanta, Georgia USA.

Autoantibody Controls, Protein Reference Unit, Sheffield, UK.

3. Principle

The AUTOZYME™ ENA method employs a unique antigen-coated microwell technology, which is ideal for the batch-screening of large numbers of samples for a range of auto-antibodies. The method utilises a non-competitive sandwich enzyme immunoassay system and is identical for all of the antigens.

First Incubation

AUTOZYME™ ENA wellstrips are provided coated with purified antigen. Serum dilutions are pipetted into the wells, allowing any antibodies present to bind to the well surface. The wells are then washed with wash buffer.

Second Incubation

Anti-IgG/IgM-alkaline phosphatase conjugate is added to the wells. Any IgG or IgM bound to the wells will bind conjugate. Any conjugate which is not specifically bound is removed by washing in wash buffer.

Third Incubation

The pale yellow substrate is added to the wells. The intensity of the pink colour formed is proportional to the concentration of auto-antibody bound in the first incubation. The reaction is stopped with the stopping buffer.

4. Kit Contents

BULK PACK (sufficient reagents for 6 Well packs)

- 2 vials wash buffer concentrate, 25 ml each
- 1 vial sample diluent concentrate, blue colour, 25 ml
- 1 vial conjugate, red colour, 22 ml
- 1 vial substrate, pale yellow colour, 22 ml
- 1 vial stopping buffer, 22 ml
- 1 96-well microplate frame (for use with wellpacks)
- 1 instruction leaflet
- 1 quality control certificate

WELL PACK

- 1 foil sachet containing 1 set of 4 x 8 single antigen-coated break-a-well microstrips, colour-coded as follows:

red - Ro (SS-A)	orange - La (SS-B)
yellow - Sm	green - Sm/RNP
blue - Jo-1	white - Scl-70
- 1 vial positive control, 1 ml lyophilised
- 1 vial calibrant, 1 ml lyophilised
- 1 quality control certificate

5. Storage

The kit should be stored refrigerated at 2°C to 8°C. Do not use the reagents beyond their expiry date. Do not freeze.

Reconstituted controls are stable for one month at -20°C. Do not exceed 4 freeze-thaw cycles.

Store unused wellstrips and desiccant in the resealable foil pouch.

Bulk Pack reagents are stable for 1 month at 2°C to 8°C after

the first use. Multiple re-use could increase the risk of reagent contamination.

Keep all reagents away from direct sunlight.

6. Safety Precautions

For *in vitro* diagnostic use only.

For Professional Use Only

In case of contact with any reagent, rinse immediately with plenty of water. Dispose of by flushing to waste with large quantities of water.

Well pack reagents contain human source material. Although such materials have been found negative when tested for HIV antibodies and hepatitis B surface antigen, no test can guarantee their absence. They should, therefore, be handled using the same safety precautions employed when handling any potentially infectious material.

Used 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 be worn when handling such items.

Safety data sheets are available on request.

7. Sample Handling

AUTOZYME™ ENA may be performed on human serum or plasma samples.

Samples should be assayed within 24 hours of collection or stored frozen at -15°C or colder. Repeated freeze-thawing of samples is not advisable.

Do not heat treat samples prior to assay.

8. Additional Reagents and Equipment

- Deionised or freshly distilled water.
- Precision micropipettes and disposable tips to deliver 10 - 1000 μ l.
- Multichannel micropipette or repeating dispenser to deliver 100 μ l.
- 200 ml and 1000 ml measuring cylinders for reagent preparation.
- Automated plate washer (optional).
- 96-well microplate reader with 540nm to 560nm filter.

9. Procedural Precautions

Allow all reagents, patient samples and control sera to equilibrate to ambient temperature (18°C to 25°C) before use. All steps must be performed at 18°C to 25°C.

Do not use reagents beyond their expiry date.

Although any Bulk pack may be used with any Well pack, reagents from different lots of Bulk packs should not be mixed, nor should reagents for different lots of Well packs.

Keep all reagents away from direct sunlight during storage and incubation.

Always interchange pipette tips between different standards, 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 yellow. Any obvious pink discoloration (absorbance >0.050 at 540-560nm) indicates that the reagent has been contaminated and must be discarded.

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

To minimise assay drift, ensure that all materials are ready to use before commencing the assay.

10. Assay Procedure

1. Let all reagents come to room temperature.
2. Prepare the following reagents:
Wash buffer: dilute the contents of each wash buffer concentrate vial to 800 ml with deionised water.
Sample diluent: dilute the contents of the sample diluent concentrate vial to 125 ml with deionised water.
Controls: reconstitute calibrant and positive control vials by adding 1 ml of deionised water and mixing gently for at least 5 minutes.
3. Dilute patient samples by 1 in 100 using the sample diluent e.g. 10 µl sample added to 990 µl diluent. Controls DO NOT require dilution.
4. Remove the antigen-coated microstrips from the foil bag, load into frame and dispense 100 µl reconstituted control or diluted sample into suitable well positions. Incubate for 30 minutes at room temperature. Seal any remaining wells in the 'whirlpak' bag, along with the desiccant sachet. It is recommended that samples be tested in duplicate.

5. Flick out the contents of the wells over a sink, gripping the frame on the long sides to retain the strips. Using the diluted wash buffer, wash the wells three times, either with an automated plate washer set to at least 300 μ l per well or by adding 300 μ l wash buffer to each well and flicking out over a sink. Alternatively use a wash bottle. Do not soak the wells for more than 1 minute. Blot the wells on absorbent material to remove any residual liquid.
6. Add 100 μ l of conjugate to each well and incubate for 30 minutes at room temperature.
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 100 μ l of substrate into each well and incubate for 30 minutes at room temperature.
9. Stop the reaction by adding 100 μ l of stopping buffer. Measure the absorbance of each well at 540nm to 560nm.

11. Treatment of Results

From a laboratory study of 100 blood donor samples, a cut-off level has been determined to be a value of 1.2 of the sample absorbance to the calibrant for each antigen. Calculate the ratio of samples to the calibrant by dividing the mean sample absorbance by the mean calibrant absorbance. Any sample giving a value of greater than 1.2 can be considered positive.

Examples:

Mean calibrant absorbance = 0.215

Sample	Mean absorbance	Value	Interpretation
1	0.155	0.7	negative
2	1.412	6.6	positive
3	0.539	2.5	positive

Refer to Bulk pack QC certificate for positive control absorbance.

Note: Interpretation of results for Sm and Sm/RNP antigens:

If the sample gives a positive result against both Sm and Sm/RNP complex, then it is interpreted as Sm positive. If the sample gives a positive result against the Sm/RNP complex only, then it is interpreted as RNP positive.

12. Quality Control

A positive control is provided within each Well pack.

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

Target performance criteria are quoted on the QC Certificate provided in the Bulk pack. If either of these criteria is not met, the results are invalid and the assay should be repeated.

13. Performance Characteristics

1. Expected values

AUTOZYME™ ENA was used to determine the auto-antibody profile of 100 blood donor plasma samples. The mean ratio plus three standard deviations gave a cut-off value of 1.2 for each antigen. It is recommended that each laboratory determines its own reference range.

2. Interfering analytes

No significant interference was detected with the following potential interferants:

bilirubin	500 mg/l
haemoglobin	5 g/l
ascorbic acid	1.0 g/l
lipids	10% (v/v)
IgG kappa myeloma serum	16 mg/ml IgG
IgM lambda myeloma serum	4 mg/ml IgM

3. Cross reactivity

Purified antigens have been used throughout. Assays have been checked with characterised antisera to ensure that there is no cross-reaction between related AUTOZYME™ ENA antigens.

4. Prozone effect

Three series of dilutions from 1/100 to 1/5 were prepared from samples with high concentration of each auto-antibody. These were run in place of the normal 1/100 diluted sample to demonstrate that high levels of auto-antibody do not fall below the cut-off to give false negatives.

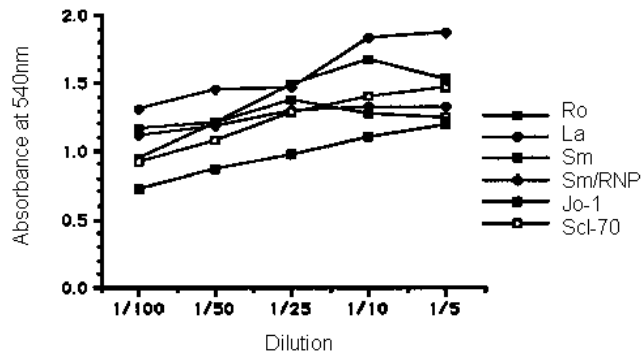


Figure 1 Graph to show effect of high concentrations of auto-antibody.

14. References

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Test procedure

