



AccuDiag™ ENA Combined Screen ELISA Kit

REF 2552

PIC AD2552ZT1

IVD See External Label 2°C 8°C 96 Tests

ENA Combined Screen ELISA

Principle	Indirect ELISA
Detection	1 – 100 U/mL
Sample	10 µL serum/plasma
Incubation Time	65 minutes
Sensitivity	1 U/mL
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation, Inc. ENA Combined Screen ELISA is a test system for the quantitative measurement of IgG class autoantibodies against extractable nuclear antigens (ENA) SS-A (52 and 60 kDa), SS-B, Sm, RNP/Sm, Scl-70, and Jo-1 in human serum or plasma.

This product is intended for professional in vitro diagnostic use only.

ASSAY PRINCIPLE

Purified antigens SS-A (52 and 60 kDa), SS-B, Sm, RNP/Sm, Scl-70 and Jo-1 are bound to individual rows A to H of the microwell plate.

The determination is based on an indirect enzyme linked immune reaction with the following steps:

Specific antibodies in the sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components.

Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution, the bound enzyme conjugate hydrolyses the substrate forming a blue colored product. Addition of an acid stops the reaction generating a yellow end-product.

The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2°C–8°C for up to five days or stored at -20°C for up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

MATERIALS AND COMPONENTS

Materials provided with the test kit

Sufficient for 96 determinations

MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use.
CONTROL A	1x 1.5 mL	Control A 12.5 U/mL, containing ENA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
CONTROL B	1x 1.5 mL	Control B 25 U/mL, containing ENA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
CONTROL C	1x 1.5 mL	Control C 50 U/mL, containing ENA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
CONTROL D	1x 1.5 mL	Control D 100 U/mL, containing ENA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
DILUENT	20 mL	Sample Buffer P , containing PBS, BSA, detergent, preservative NaN ₃ 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 mL	Enzyme Conjugate ; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative Proclin 0.05%, light red. Ready to use.
TMB	15 mL	TMB Substrate ; containing 3,3', 5,5'-Tetramethylbenzidine, colorless. Ready to use.
STOP	15 mL	Stop Solution ; contains acid. Ready to use.
WASH	20 mL	Wash Buffer , containing Tris, detergent, preservative NaN ₃ 0.09%; 50X conc.

Materials required but not provided

- Microplate reader capable for endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software

Diagnostic Automation/Cortez Diagnostics, Inc.

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Diagnostic Automation/Cortez Diagnostics, Inc.

IMMUNODIAGNOSTICS

- Multi-Channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionized water
- Measuring cylinder for 1000 mL and 100 mL
- Plastic container for storage of the wash solution

This ELISA is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

STORAGE AND STABILITY

- Store test kit at 2°C - 8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and desiccated in the clip bag provided.
- Shelf life of the unopened test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Solution and Sample Buffer are stable for at least 30 days when stored at 2°C - 8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20°C - 28°C) prior to use.
- Prepare all reagents and samples. Once started, perform the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

REAGENT PREPARATION

Wash Buffer (WASH)

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 mL prior to use.

Sample Buffer P (DILUENT)

Prior to use dilute the contents (20 mL) of one vial of sample buffer 5x concentrate with distilled or deionized water to a final volume of 100 mL.

PREPARATION OF SAMPLES

Dilute all patient samples 1:100 with sample buffer prior to use in the assay. Put 990 µL of prediluted sample buffer in a polystyrene tube and add 10 µL of sample. Mix well.

Note: Controls are ready to use and need not be diluted.

ASSAY PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

- Pipette **100 µL** of calibrators, controls and prediluted patient samples into the wells.
- Incubate for **30 minutes** at room temperature (20°C - 28°C).
- Discard the contents of the microwells and **wash 3 times with 300 µL** of wash solution.
- Dispense **100 µL** of enzyme conjugate into each well.
- Incubate for **15 minutes** at room temperature.
- Discard the contents of the microwells and **wash 3 times with 300 µL** of wash solution.
- Dispense **100 µL** of TMB substrate solution into each well.
- Incubate for **15 minutes** at room temperature.
- Add **100 µL** of stop solution to each well of the modules.
- Incubate for **5 minutes** at room temperature.
- Read** the optical density at 450 nm (reference 600-690 nm) and calculate the results. The developed color is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12	Antigens coated in rows:
A	A	P1	A	P1									Reference
B	B	P2	B	P2									Reference
C	C	P3	C	P3									SS-A
D	D	P4	D	P4									SS-B
E	E	P5	E	P5									Sm
F	F	P6	F	P6									RNP/Sm
G	C+	P7	C+	P7									Scl 70
H	C-	P8	C-	P8									Jo-1

P1, ... patient sample, A, B, C, D controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.

If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For **qualitative evaluation** the optical density (OD) of a sample is compared to the OD of Control B:

Negative:	OD sample	<	OD Control B
Positive:	OD sample	≥	OD Control B

For quantitative results plot the optical density of each control versus the control concentration to create a calibration curve. The concentration of samples may then be estimated from the calibration curve by interpolation.

Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.



PERFORMANCE CHARACTERISTICS

1. Calibration

This assay system is calibrated against the internationally recognized reference sera from CDC, Atlanta USA.

2. Measuring range

The calculation range of this ELISA assay is: 0 - 100 U/mL

3. Interpretation of results

Negative: < 25 U/mL

Positive: ≥ 25 U/mL

4. Limit of Detection

The limit of detection was determined to be: 1 U/mL

5. Interfering substances

No interference has been observed with hemolytic (up to 1000 mg/dL) or lipemic (up to 3 g/dL triglycerides) sera or plasma, or bilirubin (up to 40 mg/dL) containing sera or plasma.

Nor have any interfering effects observed with the use of anticoagulants (Citrate, EDTA, Heparin).

However, for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum-based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3',5,5'-Tetramethyl-benzidine).
- Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:
- First aid measures:
In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:
Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- Exposure controls / personal protection:

Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.

11. Conditions to avoid:

Since substrate solution is light-sensitive. Store in the dark.

12. For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

MANUFACTURER AND BRAND DETAILS

 ISO 13485:2016 ISO 13485 Quality Management for Medical Devices CERTIFIED	
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