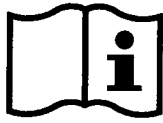


# Product information



Instruction for use



Distribuito in ITALIA da  
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## ENA Combi ELISA

Enzyme Immunoassay for semi-quantitative screening for IgG Extractable Nuclear Antigens in human serum or plasma



DE7390



96 Tests

**PRINCIPLE OF THE TEST**

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. Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

**SUMMARY AND EXPLANATION OF THE TEST**

Connective tissue diseases (CTD) are a group of autoimmune disorders which are characterized by presence of antinuclear antibodies (ANA) in the blood of patients. ANA are a specific class of autoantibodies that have the capability of binding and destroying certain structures within the nucleus of the cells. These antibodies are involved in the disease pathogenesis, and they also constitute the basis for diagnosis and treatment of CTD. ANA have been categorized into two main groups:

1. Autoantibodies to DNA and histones
2. Autoantibodies to extractable nuclear antigens (ENA): Sm, ribonucleoproteins (RNP), SSA/Ro, SSB/La, Scl-70, Jo-1 and PM1 Autoantibodies to DNA and histones include antibodies against single and double stranded DNA (ssDNA and dsDNA).

Significant levels of anti-dsDNA antibodies are considered to be confirmatory in the diagnosis of systemic lupus erythematosus (SLE). Anti-histone antibodies are indicative of drug-induced lupus.

Besides DNA and histones, autoantibodies may also target other nuclear antigens. These nuclear antigens were named extractable nuclear antigens (ENA), as originally they were extracted from the nuclei with saline solution. Autoantibodies to Smith antigen (Sm) which is also considered to be highly specific for SLE were the first anti-ENA detected. Thereafter, further subtypes of ENA i.e. ribonucleoproteins (RNP), Sjögren antigen A or B (SSA/Ro or SSB/La), Scl-70, Jo-1 and PM1 were identified. Although most of these ENA are disease specific, a significant overlap exists. Sensitivity and specificity may also vary depending upon the type of underlying CTD.

Presence of autoantibodies in the sera of patients constitutes one of the criteria used for diagnosis of CTD. Together with the clinical diagnosis ANA subtyping helps in identifying a specific CTD.

Indirect immunofluorescence tests (IF) and enzyme immunoassays (ELISA) are commonly used for ANA detection in day to day practice. Initially, screening is carried out by IF-ANA or a generic ELISA which detects ANA of a broad specificity similar to IF-ANA. If positive, more specific tests are performed based on clinical findings and the IF-ANA staining pattern. These antigen specific ELISA assays react with single autoantigens e.g. dsDNA, SS-A/Ro, SS-B/La, Scl-70, Sm, Sm/RNP etc.

Autoantibodies to dsDNA are specific and diagnostic for SLE and levels are elevated during active disease. Recently published ACR Guidelines for Screening, Treatment, and Management of Lupus Nephritis recommend the testing of antibodies to dsDNA for monitoring of lupus nephritis, ranging from monthly intervals in pregnant patients with active glomerulonephritis at onset of treatment to every three months in patients with active nephritis at onset of treatment or pregnant patients with previous but not current nephritis, up to six-monthly testing in patients with previous active nephritis or no prior or current nephritis.

SLE-Patients without antibodies against dsDNA often produce antibodies against ssDNA. Similarly anti-Sm is highly specific for SLE but is present in only 10% to 30 % of SLE cases.

Antibodies against dsDNA, histones, the 70 kD protein of the U1-snRNP complex (RNP70) and anti Sm are closely associated with SLE. Anti-SSA/Ro and anti-SSB/La antibodies are indicative for Sjögren's syndrome, but can also be found in up to 30 % cases of SLE with cutaneous involvement.

Anti-SS-A/Ro antibodies pass the placenta and may cause the development of SLE in neonates. Anti-SSA/Ro antibodies are almost always present in sera of mothers with babies with neonatal lupus syndrome and with complete congenital heart block.

Antinucleolar antibodies are a group of autoantibodies which give a nucleolar IF-staining pattern. Most common of these are anti-PM-Scl, anti-RNA polymerase I-III and anti-U3-RNP They are found in scleroderma and polymyositis (PM). Antibodies against RNP and the complex RNP/Sm are linked to mixed connective tissue disease (MCTD, Sharp syndrome) and to SLE. Serologically MCTD is characterized by the presence of autoantibodies directed against the 70 kD protein of the U1-snRNP-complex. Up to 100% of MCTD patients manifest high titers of Anti-RNP-70 antibodies.

**Autoantibody prevalence to (values in %)**

| Diseases  | dsDNA | ssDNA | Histone | SS-A  | SS-B  | Sm    | RNP/ Sm | Scl-70 | Jo-1  |
|---|-------|-------|---------|-------|-------|-------|---------|--------|-------|
| Systemic lupus erythematosus (SLE)              | > 90  | > 90  | 30-50   | 10-30 | 30-50 | 10-30 | 10-30   |        |       |
| Drug-induced lupus (DIL)                        |       | 30-50 | 50-90   |       |       |       |         |        |       |
| Sharp-syndrome/ mixed connective tissue disease | 10-30 | 10-30 |         |       |       |       | > 90    |        |       |
| Rheumatoid arthritis                            | 10-30 | 30-50 | 30-50   | 10-30 |       |       |         |        |       |
| Sjögren's syndrome                              | 10-30 | 10-30 |         | > 90  | > 90  |       |         |        |       |
| Scleroderma                                     | 10-30 | 10-30 |         | 10-30 |       |       |         | > 90   |       |
| Photosensitive dermatitis, dermatomyositis      | 10-30 | 10-30 |         |       |       |       |         |        | 50-90 |

**CONTENTS OF THE KIT**

Sufficient for 96 determinations

**1 One divisible microplate** consisting of 12 modules of 8 wells each. Ready to use.

**4x 1.5 ml Control A-D** (12.5, 25, 50, 100 U/ml), containing ENA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN<sub>3</sub> 0.09%), yellow. Ready to use

**20 ml Sample Buffer P**, containing PBS, BSA, detergent, preservative NaN<sub>3</sub> 0.09%, yellow, 5 x conc.

**15 ml Enzyme Conjugate** containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.

**15 ml TMB Substrate**; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.

**15 ml Stop solution**; contains acid. Ready to use.

**20 ml Wash Solution**, containing Tris, detergent, preservative NaN<sub>3</sub> 0.09%; 50x conc.

**1 Instruction for Use**

**1 Certificate of Analysis**

**MATERIALS REQUIRED**

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- 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 deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay 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.

**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 2-8 °C for up to five days or stored at -20 °C 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.

## STORAGE AND STABILITY

- Store test kit at 2-8 °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.
- 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 2-8 °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 (20-28 °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 ofw solution.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

## 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, calibrators, sample buffer and Wash solution contain sodium azide (NaN<sub>3</sub>) 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 nitrile rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- 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.

**PREPARATION OF REAGENTS****Wash Solution**

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

**Sample Buffer P**

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

**Preparation of samples**

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

**TEST PROCEDURE**

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

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
2. Incubate for **30 minutes** at room temperature (20-28 °C).
3. Discard the contents of the microwells and **wash 3 times with 300 µl** of wash solution.
4. Dispense **100 µl** of enzyme conjugate into each well.
5. Incubate for **15 minutes** at room temperature.
6. Discard the contents of the microwells and **wash 3 times with 300 µl** of wash solution.
7. Dispense **100 µl** of TMB substrate solution into each well.
8. Incubate for **15 minutes** at room temperature
9. Add **100 µl** of stop solution to each well of the modules
10. Incubate for **5 minutes** at room temperature.
11. Read the optical density at 450 nm (reference 600-690nm) and calculate the results. The developed colour 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  | B  | C  | D  |   |   |   |   |   |    |    |    | Reference                |
| B | A  | B  | C  | D  |   |   |   |   |   |    |    |    | Reference                |
| C | P1 | P2 | P3 | P4 |   |   |   |   |   |    |    |    | SS-A                     |
| D | P1 | P2 | P3 | P4 |   |   |   |   |   |    |    |    | SS-B                     |
| E | P1 | P2 | P3 | P4 |   |   |   |   |   |    |    |    | Sm                       |
| F | P1 | P2 | P3 | P4 |   |   |   |   |   |    |    |    | RNP/Sm                   |
| G | P1 | P2 | P3 | P4 |   |   |   |   |   |    |    |    | Sci-70                   |
| H | P1 | P2 | P3 | P4 |   |   |   |   |   |    |    |    | 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 calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient 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****Calibration**

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

**Measuring range**

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

**Expected values**

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 25 U/ml

**Interpretation of results**

Negative: < 25 U/ml

Positive: ≥ 25 U/ml

**Linearity**

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper/ lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

| Sample   | Dilution | Observed U/ml | Expected U/ml | O/E [%] |
|----------|----------|---------------|---------------|---------|
| (SS-A)   | 1:100    | 95.0          | 95.0          | 100     |
| .        | 1:200    | 45.6          | 47.5          | 96      |
| .        | 1:400    | 22.5          | 23.8          | 95      |
| .        | 1:800    | 12.1          | 11.9          | 102     |
| (SS-B)   | 1:100    | 62.5          | 62.5          | 100     |
| .        | 1:200    | 33.0          | 31.3          | 106     |
| .        | 1:400    | 16.8          | 15.6          | 108     |
| .        | 1:800    | 9.0           | 7.8           | 115     |
| (Sm)     | 1:100    | 80.6          | 80.6          | 100     |
| .        | 1:200    | 39.5          | 40.3          | 98      |
| .        | 1:400    | 20.9          | 20.2          | 104     |
| .        | 1:800    | 10.2          | 10.1          | 101     |
| (RNP/Sm) | 1:100    | 88.0          | 88.0          | 100     |
| .        | 1:200    | 41.2          | 44.0          | 94      |
| .        | 1:400    | 21.0          | 22.0          | 95      |
| .        | 1:800    | 10.6          | 11.0          | 96      |
| (Scl-70) | 1:100    | 70.4          | 70.4          | 100     |
| .        | 1:200    | 36.1          | 35.2          | 103     |
| .        | 1:400    | 18.0          | 17.6          | 102     |
| .        | 1:800    | 9.0           | 8.8           | 102     |
| (Jo-1)   | 1:100    | 98.0          | 98.0          | 100     |
| .        | 1:200    | 48.2          | 49.0          | 98      |
| .        | 1:400    | 24.0          | 24.5          | 98      |
| .        | 1:800    | 11.8          | 12.3          | 96      |

**Limit of detection**

Functional sensitivity was determined to be: 1 U/ml

**INTERFERING SUBSTANCES**

No interference has been observed with haemolytic (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 been observed with the use of anticoagulants (Citrates, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

**Reproducibility**

*Intra-assay precision:* Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

*Inter-assay precision:* Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

| Intra-Assay |           |      | Inter-Assay |           |      |
|-------------|-----------|------|-------------|-----------|------|
| Sample      | Mean U/ml | CV % | Sample      | Mean U/ml | CV % |
| SS-A        | 30.5      | 4.0  | SS-A        | 31.2      | 4.3  |
| SS-B        | 28.4      | 3.8  | SS-B        | 29.5      | 4.1  |
| Sm          | 26.9      | 2.1  | Sm          | 27.3      | 2.6  |
| RNP/Sm      | 28.9      | 3.2  | RNP/Sm      | 27.4      | 3.8  |
| Sci-70      | 26.8      | 2.6  | Sci-70      | 25.7      | 2.6  |
| Jo-1        | 25.0      | 2.0  | Jo-1        | 25.4      | 2.5  |

**Study results**

| Study population      | n   | n Pos* | %     |
|-----------------------|-----|--------|-------|
| SLE                   | 63  | 57     | 90.5  |
| Sjögren's Syndrome    | 10  | 10     | 100.0 |
| MTCO                  | 10  | 10     | 100.0 |
| Scleroderma           | 10  | 10     | 100.0 |
| Poly-Dermatomyositis  | 8   | 7      | 87.5  |
| Rheumathoid arthritis | 60  | 2      | 3.3   |
| Normal human sera     | 148 | 3      | 2.0   |

\*Positive for one or more antigens

Clinical Diagnosis

|     | Pos | Neg |     |
|-----|-----|-----|-----|
| Pos | 94  | 5   |     |
| Neg | 7   | 203 |     |
|     | 101 | 208 | 309 |

Sensitivity: 93.1 %

Specificity: 97.6 %

Overall agreement: 96.1 %

**LIMITATIONS OF THE PROCEDURE**











This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually. The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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## SYMBOLS USED WITH DEMEDITEC ASSAYS

| Symbol  | English                            | Deutsch                      | Français                                 | Español                               | Italiano                            |
|---|------------------------------------|------------------------------|--|---------------------------------------|-------------------------------------|
|  | Consult instructions for use       | Gebrauchsanweisung beachten  | Consulter les instructions d'utilisation | Consulte las instrucciones de uso     | Consultare le istruzioni per l'uso  |
|  | European Conformity                | CE-Konformitätskennzeichnung | Conformité aux normes européennes        | Conformidad europea                   | Conformità europea                  |
|  | In vitro diagnostic device         | In-vitro-Diagnostikum        | Usage Diagnostic in vitro                | Para uso Diagnóstico in vitro         | Per uso Diagnostica in vitro        |
|  | For research use only              | Nur für Forschungszwecke     | Seulement dans le cadre de recherches    | Sólo para uso en investigación        | Solo a scopo di ricerca             |
|  | Catalogue number                   | Katalog-Nr.                  | Numéro de catalogue                      | Número de catálogo                    | Numero di Catalogo                  |
|  | Lot. No. / Batch code              | Chargen-Nr.                  | Numéro de lot                            | Número de lote                        | Numero di lotto                     |
|  | Contains sufficient for <n> tests/ | Ausreichend für "n" Ansätze  | Contenu suffisant pour "n" tests         | Contenido suficiente para <n> ensayos | Contenuto sufficiente per "n" saggi |
|  | Storage Temperature                | Lagerungstemperatur          | Température de conservation              | Temperatura de conservación           | Temperatura di conservazione        |
|  | Expiration Date                    | Mindesthaltbarkeitsdatum     | Date limite d'utilisation                | Fecha de caducidad                    | Data di scadenza                    |
|  | Legal Manufacturer                 | Hersteller                   | Fabricant                                | Fabricante                            | Fabbricante                         |
| Distributed by  | Distributor                        | Vertreiber                   | Distributeur                             | Distribuidor                          | Distributore                        |
| Content   | Content                            | Inhalt                       | Conditionnement                          | Contenido                             | Contenuto                           |
| Volume/No.  | Volume / No.                       | Volumen/Anzahl               | Volume/Quantité                          | Volumen/Número                        | Volume/Quantità                     |