ENA and CENP B IgG ELISA Kit

Catalog Number KA6331

96 tests

Version: 01

Intended for research use only

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Introduction

Intended Use

Enzyme immunoassay for the detection of IgG antibodies to individual extractable nuclear antigens and centromere B in human serum or plasma.

Background

Determination of antinuclear antibodies is important tool for differential diagnosis of systemic autoimmune diseases. Antinuclear antibodies are autoantibodies directed against intracellular antigens located mainly in the cell nucleus or the cytoplasm. Their detection can indicate the presence of systemic autoimmunopathologic process. Related disorders include systemic lupus erythematodes (SLE), Sjögren's syndrome (SS), sclerodermia, mixed connective tissue disease (MCTD), systemic sclerosis, polymyositis and dermatomyositis.

Extractable nuclear antigens (ENA) are a group of antinuclear antigens: SS-A/Ro, SS-B/La, Sm, RNP, Scl-70 and Jo-1. They are mainly ribonucleoproteins and nuclear enzymes. Antibodies against SS-A/Ro and SS-B/La often occur in patients with SS and SLE. Anti-Sm antibodies represent a highly specific marker, and one of diagnostic and classification criteria for SLE. Anti-RNP antibodies (a part of Sm/RNP complex) are also often detected in patients with SLE. Presence of these antibodies is highly specific for MCTD (particularly when anti-Sm antibodies are missing). Detection of anti-Jo-1antibodies is significant for another group of organ non-specific autoimmune diseases – myositis. Antibodies against antigen Scl-70 are important diagnostic markers for systemic sclerosis (particularly its progressive forms).
The group of antinuclear antibodies also includes antibodies against nucleic acids (ssDNA, dsDNA), complexes of nuclear proteins (DNP, RNP) and histones.

Principle of the Assay

The kit is intended for detection of specific IgG antibodies in a sample by means of a sandwich type of the EIA method (i.e. a solid phase coated with specific antigen – antibody from the analysed sample – labelled antibody). The labelled antibody (conjugate) is an animal immunoglobulin fraction to human IgG conjugated with horseradish peroxidase. Peroxidase activity is determined in the test by a substrate containing TMB. Positivity is indicated when blue colour appears; after stopping solution has been added, blue changes to yellow. The yellow colour intensity is measured by a photometer at 450 nm, and it is proportional to the concentration of specific IgG antibodies in the sample.

Antigen used:

Mixture of native and recombinant antigens: Ro52/SS-A, Ro60/SS-A, La/SS-B, RNP-A, RNP-C, RNP 68, Sm, Scl-70, Jo-1 and centromere B

General Information

Materials Supplied

List of component

Component	Amount
Microtitre Plate (MICROPLATE): Coated with antigen, 12 x 8 wells in bag with desiccant.	1 pc
Negative Control (CONTROL -): solution containing no specific human antibodies, ready to use.	2 mL
CUT-OFF(CUTOFF): solution containing specific human antibodies in cut-off concentration, ready to use.	2 mL
Positive Control (CONTROL +): solution containing specific human antibodies, ready to use.	2 mL
Conjugate (CONJUGATE): solution containing peroxidase labelled animal immunoglobulin to human IgG, ready to use.	15 mL
Sample Diluent 2 (DILUENT 2): Buffer with protein stabilizers, ready to use.	105 mL
TMB-Complete 2 (SUBSTRATE 2): Chromogenic substrate solution containing TMB/H_2O_2, ready to use.	15 mL
Wash Solution (WASH 20x): 20x concentrated buffer.	75 mL
Stop Solution (STOP): Acid solution, ready to use.	15 mL

Storage Instruction

Store the kit at +2°C to +8°C. Do not freeze. If the kit is stored as described, the labelled expiration date is valid. The expiration date is indicated on the package. The opened kit should be used within three months.

Materials Required but Not Supplied

- ✓ Single and multichannel pipettes
- ✓ Disposable tips
- ✓ Microplate washer
- ✓ Timer
- ✓ Incubator (37°C)
- ✓ Microplate reader

Precautions for Use

✓ Safety Precautions

1. The kit is intended for research use only.
2. The sera used for controls were tested and found to be negative for HIV 1 and HIV 2, HBsAg, HCV, TPHA. In spite of this fact, they still need to be handled as potentially infectious materials.
3. Some reagents contain sodium azide, which is a toxic compound. Avoid contact with skin.
4. The Stop Solution contains diluted acid solution. Avoid contact with eyes and skin.
5. It is necessary to observe the local safety rules and regulations.

- First aid

  In case of contact with eyes, flush with copious amount of water and seek medical assistance. In case of contact with skin and clothing, remove all the contaminated clothes. Wash the skin with soap and plenty of running water. In case of contact with solutions containing plasma or samples, disinfect the skin. In case of accidental ingestion, flush the mouth with drinking water and seek medical assistance.
- Remnants disposal

  All the materials used for performing the test must be treated as potentially infectious due to the contact with biological materials. Therefore they need to be disposed together with biological waste.
- Expired kit disposal

  Disassemble the kit and dispose the components as biological material. Discard the packaging material as required by local regulations.

✓ Procedural Notes

1. In order to obtain reliable results, it is necessary to strictly follow the protocol. Always use clean preferably disposable tips and glassware.
2. Microtitre Plate: in order to prevent water condensation on the surface of the microplate, always allow the bag with the microplate to warm up to room temperature before opening.
3. Wash Solution: use high quality distilled water for preparing the working strength Wash Solution.
4. Washing procedure: keep to the prescribed number of wash cycles and fill the wells to the upper edge. The soak time (i.e. interval between two different wash cycles during which the wells stay filled up with the Wash Solution) should be approx. 30-60 seconds.
5. TMB-Complete: the vessel used for multichannel pipetting should not be used for other reagents. Do not return the surplus TMB-Complete from the pipetting vessel into the vial.
6. Non-reproducible results might be caused by improper methodology as following:

- Insufficient mixing of reagents and samples before use.
- Improper replacement of vial caps.
- Using the same tip for pipetting different reagents.
- Reagent exposure to excessive temperature; bacterial or chemical contamination.
- Insufficient washing or filling of the wells (the wells should be filled to the upper edge), improper aspiration of Wash Solution remnants.
- Contamination of the well edges with Conjugate or samples.
- Using reagents from different kit lots.
- Contact of reagents with oxidants, heavy metals and their salts.

7. The kit might be used for sequential examinations. When preparing working strength solutions, use only the amount of reagents needed for the analysis.
8. The kit might be used in all types of automatic EIA analyzers.
9. The producer cannot guarantee that the kit will function properly if the assay procedure instructions are not strictly adhered to.

Assay Protocol

Reagent Preparation

- ✓ Dilute the Wash Solution 1:20 (1 part of solution and 19 parts of distilled water); e.g. 75 mL of the concentrated Wash Solution + 1425 mL of distilled water.
  Salt crystals might develop in the bottle with the concentrated Wash Solution. Prior to use, it is necessary to dissolve the crystals by warming the bottle in a water bath. The diluted Wash Solution is stable at +2°C to +8°C for one week.
- ✓ The Controls (positive, negative and CUT-OFF) are ready to use, do not dilute further!
- ✓ The Conjugate is ready to use, do not dilute further!
- ✓ TMB-Complete is a one-component chromogenic substrate solution ready to use, do not dilute further!

Sample Preparation

Mix gently the Sample Diluent prior to use.

- ✓ Sample Preparation and Storage
- The following human body liquids can be used for testing: serum and citrate plasma.
- Anticoagulants in the plasma (except for citrate) as well as bacterially contaminated, haemolytic or chylous samples can affect the test results.
- Samples can be stored at +2°C to +8°C for one week. For a longer period, store samples at -20°C.
- Diluted samples should be used as soon as possible.

- ✓ Dilution of sera and plasma samples
  Dilute well mixed samples 1:101 with the Sample Diluent:
  E.g.: 10 µL of sample + 1 mL of the Sample Diluent
  Mix well.

Assay Procedure

✓ Allow all reagents to come to room temperature and mix well. If you do not use a whole microplate, return unnecessary strips into the bag with desiccant. Seal the bag tightly and store at +2°C to +8°C. Keep dry!

1. Dispense the controls and the diluted samples according to the Plate Layout.

- Leave A1 well empty (blank).
- Pipette 100 µL of the Negative Control into 1 well..
- Pipette 100 µL of the CUT-OFF 2 wells..
- Pipette 100 µL of the Positive Control to 1 well.
- Pipette 100 µL of the diluted samples (see Section Sample Preparation) into the other wells.

2. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
3. Aspirate the content of the wells and wash 5x with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
4. Pipette 100 µL of the Conjugate into all wells except A1 well.
5. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
6. Aspirate the content of the wells and wash 5x with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
7. Pipette 100 µL of TMB-Complete into all wells. Avoid contamination (see Procedural Notes section).
8. Cover the microplate with the lid and incubate at 37°C for 15 minutes. Keep out of light.
9. Stop the reaction by adding 100 µL of the Stop Solution in the same order and intervals as the substrate was added.
10. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction.

Data Analysis

Calculation of Results

✓ Quality Control

- The test is valid if:

The absorbance of blank is lower than 0.150.

Blank < 0.150

The absorbance of the Negative Control is lower than half of the mean absorbance of CUT–OFF.

(Control -) < 0.5 x (CUTOFF)

The mean absorbance of CUT-OFF is within a range of 0.200 - 1.000.

0.200 < (CUTOFF) < 1.000

The absorbance of the Positive Control is 1.5-fold higher than the mean absorbance of CUT–OFF.

(Control +) > 1.5 x (CUTOFF)

✓ Results Interpretation

- Calculation of Index of Positivity (IP)

Divide the absorbance of a tested sample by the mean absorbance of CUT-OFF measured in the same test run:

$$IP = \frac{\text{Absorbance of sample}}{\text{Mean absorbance of CUT-OFF}}$$

Interpretation of the test results is described in Table 1.

Table 1: Interpretation of test results

Index of Positivity (IP)	Evaluation
lower than 0.9	negative
0.9 to 1.1	borderline
higher than 1.1	positive

Examination of borderline samples, i.e. samples with Index of Positivity from 0.9 to 1.1, should be repeated from a new sample collected after certain time regarding to the disease specifics.

Resources

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Diluted tested sample 4										
B	Negative Control	Diluted tested sample 5										
C	CUTOFF											
D	CUTOFF											
E	Positive Control											
F	Diluted tested sample 1											
G	Diluted tested sample 2											
H	Diluted tested sample 3											