

**User's Manual**

ANA IgG Screening ELISA Kit

REF DEIABL567 Σ 96T**RUO**

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The ANA Screen ELISA test system is an enzyme-linked immunosorbent assay (ELISA) for the detection of IgG class antibodies to ANA in human serum or plasma. For research use only.

Principles of Testing

Diluted serum is added to wells coated with purified nuclear antigens. If present, the ANA IgG specific antibody binds to the antigen. All unbound materials are then washed away, and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off, and substrate is added. The plate is then incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

Reagents And Materials Provided

1. Microwell coated with nuclear antigens 12x8x1
2. Sample Diluent: 1 bottle (ready to use) 22 mL
3. Calibrator 1 Vial (ready to use) 1 mL
4. Positive Control 1 vial (ready to use) 1 mL
5. Negative Control 1 vial (ready to use) 1 mL
6. Enzyme conjugate: 1 bottle (ready to use) 12 mL
7. TMB Substrate: 1 bottle (ready to use) 12 mL
8. Stop Solution: 1 bottle (ready to use) 12 mL
9. Wash concentrate 20x: 1 bottle: 1 bottle 25 mL

Materials Required But Not Supplied

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper

Storage

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

4. Do not expose test reagents to heat, sun or strong light.

Specimen Collection And Preparation

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2-8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

Reagent Preparation

Prepare 1x Wash buffer by adding the contents of the bottle (25 ml, 20x) to 475 ml of distilled or deionized water. Store at room temperature (18-26 °C).

Assay Procedure

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µL of 1x wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1x wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

Quality Control

The test run may be considered valid provided the following criteria are met:

1. If the O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab index for Positive control should be greater than 1.2.

Calculation

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Reference Values

Example of typical results:

Calibrator mean OD = 0.8

Calibrator Factor (CF) = 0.5

Cut-off Value = $0.8 \times 0.5 = 0.400$

Positive control O.D. = 1.2

Ab Index = $1.2 / 0.4 = 3$

Sample O.D. = 1.6

Ab Index = $1.6 / 0.4 = 4.0$

Performance Characteristics

354 sera were tested by this ELISA and a reference ELISA methods. 148 sera were positive and 188 sera were negative by both methods. The agreement between the two methods was 95% (336/354). The results are summarized below.

	ANA IgG ELISA				Total
	+	+/-	-		
Reference ELISA	+	148	6	4	158
Kit	+/-	2	0	0	2
	-	6	0	188	194
Total		156	6	214	154

Precision

Intra-assay

Serum	Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.41	0.6	4.22
2	16	0.82	0.2	2.6
3	16	0.29	0.3	9.48

Inter-assay

Serum	Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.15	0.09	7.43
2	10	0.81	0.1	11.8
3	10	0.29	0.03	9.48

Precautions

1. Potential biohazardous materials:

The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.

1. This kit is designed for research use only.
2. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
3. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

Limitations

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1. The test results obtained using this kit are for research use only.
2. Lipemic or hemolyzed samples may cause erroneous results.