DRG® Anti-Nuclear Antibodies (ANA) Screen (EIA-4830)

As of 9 Feb. 2010 rm (Vers. 1.1) **USA: RUO**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

The ANA Screen ELISA test system is an enzyme linked immunosorbent assay (ELISA) for the detection of IgG antibodies to anti-nuclear antibodies (ANA) in human serum.

PRINCIPLE OF THE TEST

Diluted serum is added to wells coated with purified nuclear antigens. ANA IgG or IgM specific antibody, if present, binds to the antigen. All unbound materials are 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 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.

MATERIALS PROVIDED

1. Microwell coated with nuclear antigens 12x8
2. Sample Diluent: 1 bottle (ready to use) 22 mL
3. Calibrator: yellow Cap. 1 Vial (ready to use) 1.5 mL
4. Positive Control: Red Cap. 1 vial (ready to use) 1.5 mL
5. Negative Control: Blue Cap. 1 vial (ready to use) 1.5 mL
6. Enzyme conjugate: 1 bottle (ready to use) 12 mL
7. TMB Substrate: 1 bottle (ready to use) 12 mL
8. Stop Solution: 1N HCL, 1 bottle (ready to use) 12 ml
9. Wash concentrate 20X: 1 bottle 25 mL

MATERIALS NOT PROVIDED

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

STORAGE AND STABILITY

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.

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WARNINGS AND 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
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 pipette by mouth. 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.

SPECIMEN COLLECTION AND HANDLING

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 RT.

PREPARATION FOR ASSAY

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

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. ***Negative control, positive control, and calibrator are ready to use.***
3. Prepare 1:21 dilution of test samples, by adding 10 µL of the sample to 200 µL of sample diluent. Mix well.
4. Dispense 100 µL of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µL 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.
5. Remove liquid from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on absorbance paper or paper towel.
6. Dispense 100 µL of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
7. Remove enzyme conjugate from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on absorbance paper or paper towel

8. Dispense 100 µL of TMB substrate and incubate for 10 minutes at room temperature.
9. Add 100 µL of stop solution.
10. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

CALCULATION OF RESULTS

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.

EXAMPLE OF TYPICAL RESULTS:

Calibrator mean OD	=	0.8
Calibrator Factor (CF)	=	0.5
Cut-off Value = 0.8 x 0.5	=	0.400
Positive control O.D.	=	1.2
Ab Index = 1.2 / 0.4	=	3
Specimen sample O.D.	=	1.6
Ab Index = 1.6 / 0.4	=	4.0

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