

**MBL**

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## **Anti-RNA Polymerase III ELISA Kit**

Cat. No. 7805E: 96 wells

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## English

### **INTENDED USE**

The anti-RNA Polymerase III ELISA Kit is a semi-quantitative, enzyme-linked immunosorbent assay (ELISA) for the detection of anti-RNA Polymerase III antibodies in human serum. The anti-RNA Polymerase III ELISA Kit is intended for in vitro diagnostic use as an aid in the determination of certain autoimmune diseases.

### **SUMMARY AND EXPLANATION**

Systemic sclerosis (SSc) is an autoimmune disease characterized by microvascular damage and fibrosis of the skin and internal organs<sup>(1)</sup>. RNA polymerase (RNAP) I, II and III are major targets of autoantibody responses in SSc patients. Each RNAP catalyzes transcription of unique sets of genes: RNAP I transcribes ribosomal RNA genes, RNAP II transcribes all protein coding genes and several small nuclear RNA genes, and RNAP III transcribes genes that produce small stable RNAs including 5S and transfer RNAs<sup>(2)</sup>. Anti-RNAP I and anti-RNAP III antibodies are almost always present together (anti-RNAP I/III), and some sera contain anti-RNAP II antibody as well<sup>(3-5)</sup>. Anti-RNAP I/III antibodies are the most common SSc related antibodies in white North American patients with SSc<sup>(6)</sup> and is associated with diffuse cutaneous involvement, a high frequency of “renal crisis”, and high mortality<sup>(3,4,7)</sup>. In addition, it has been shown that some SSc sera contain autoantibodies recognizing RNAP II but not RNAP I or III. Antibodies to RNAP II are also present in sera from some patients with systemic lupus erythematosus (SLE) or overlap syndrome<sup>(8,9)</sup>.

The anti-RNA Polymerase III ELISA Kit measures with high sensitivity, anti-RNA Polymerase III antibodies present in the serum by ELISA.

### **PRINCIPLE**

The anti-RNA Polymerase III ELISA Kit measures anti-RNAP III antibodies present in the serum by ELISA. Diluted Calibrators and patient serum are added to microwell coated with RNAP III antigens, allowing anti-RNAP III antibodies to react with the immobilized antigen (Sample incubation). After washing to remove any unbound serum proteins, horseradish peroxidase conjugated anti human IgG is added and incubated (Conjugate incubation). Following another washing step, the peroxidase substrate is added and incubated for an additional period of time (Substrate incubation). Acid solution is then added to each well to terminate the enzyme reaction and to stabilize the color development. The assay can be quantified by measuring the reaction photometrically and plotting the results.

### **BRIEF ASSAY PROCEDURE**

<Sample incubation>	Add 100 µl of diluted sample (1:101) to each well of microwell plate
(20-25 °C) 60 min.	↓
	Wash
	↓
<Conjugate incubation>	Add 100 µl of conjugate solution to each well
(20-25°C) 60 min.	↓
	Wash
	↓

<Substrate incubation>    Add 100  $\mu$ l of substrate to each well  
(20-25 °C) 30 min            ↓  
   Add 100  $\mu$ l of stop solution to each well  
   ↓  
   Read absorbance  
   ↓  
   Interpretation of result

## **REAGENTS AND STORAGE**

### **1) MICROWELL STRIPS**

96 wells MICROWELL STRIPS (12 x 8 wells) coated with antigen produced from recombinant purified proteins, the breakaway strips packaged in a strip holder and sealed in a foil envelope with desiccant, are stable at 2-8°C until the labeled expiration date.

### **2) CALIBRATOR 1 (0U/ml)**

One vial containing 1.5 ml of Assay Diluent including 0.09% sodium azide. Ready to use, make no further dilution. Stable at 2-8°C until labeled expiration date.

### **3) CALIBRATOR 2 (100U/ml)**

One vial containing 1.5 ml each of anti-RNA Polymerase III antibody positive human serum with Assay Diluent including 0.09% sodium azide. Ready to use, make no further dilution. Stable at 2-8°C until labeled expiration date.

### **4) CONJUGATE REAGENT**

One vial containing 15 ml of horseradish peroxidase conjugated goat anti-human IgG, HEPES, Proclin 150 and BSA. Ready to use. Stable at 2-8°C until labeled expiration date.

### **5) ASSAY DILUENT**

Two 50 ml bottles containing PBS, Tween-20 and 0.09% sodium azide. Ready to use. Stable at 2-8°C until labeled expiration date.

### **6) WASH CONCENTRATE (10X)**

One 100 ml bottle containing PBS and Tween-20 as a 10X concentrate. Stable at 2-8°C until labeled expiration date.

### **7) SUBSTRATE SOLUTION**

One 20 ml bottle containing 3,3',5,5'-tetramethylbenzidine dihydrochloride/hydrogen peroxide (TMB/ H<sub>2</sub>O<sub>2</sub>). Ready to use. Stable at 2-8°C until labeled expiration date.

### **8) STOP SOLUTION**

One 20 ml bottle containing 1N Sulfuric acid. Ready to use. Stable at 2-8°C until labeled expiration date.

### **9) POSITIVE CONTROL**

One vial containing 1.5 ml each of anti-RNA Polymerase III antibody positive human serum with Assay

Diluent including 0.09% sodium azide. Ready to use, make no further dilution. Stable at 2-8°C until labeled expiration date.

## 10) NEGATIVE CONTROL

One vial containing 1.5 ml of Assay Diluent including 0.09% sodium azide. Ready to use, make no further dilution. Stable at 2-8°C until labeled expiration date.

## PRECAUTIONS

- (1) This product is for in vitro diagnostic use only.
- (2) Do not use kit components beyond the stated expiration dates.
- (3) Avoid contact of reagents with eyes, skin and clothing. Reagents on skin must be washed away with plenty of water. TMB contains irritant and Stop Solution consists of a 1N sulfuric acid, which is a poison and corrosive.
- (4) Calibrator 2 and Positive control are derived from human serum, in which HBs antigen, HCV antibody HIV-1 and HIV-2 antibodies has not been detected. No test method, however, can guarantee the absence of these or any other infectious agents. These reagents and all patient samples should be handled as if they are capable of transmitting AIDS, hepatitis or any other infectious diseases.
- (5) Calibrator 1, Calibrator 2, Positive control, Negative control and Assay Diluent contain sodium azide (0.09%) as a preservative and must be handled with caution - do not ingest or allow contact with skin or mucous membranes. Sodium azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush with plenty of water when disposing materials containing sodium azide into a drain.
- (6) Some kit components contain animal origin materials, which are from non-infectious animals. These components, however, should be treated as potential biohazards in use and for disposal.
- (7) Matching lot numbers of Microwell strips, Conjugate, Assay Diluent and Calibrator 2 must be used together in the assay. Do not substitute reagents from other kits.
- (8) All reagents must be brought to room temperature (20 -25 °C) before starting the assay.
- (9) Do not expose the kit to direct sun during assay and storage.
- (10) Avoid microbial and cross contamination of reagents or samples.
- (11) Incubation temperatures above or below normal room temperature (20-25 °C), shorter or longer time periods of incubation and inaccurate dilution may give erroneous results.
- (12) The wells must be rinsed with Wash Solution properly enough to avoid false positive.
- (13) Carefully pipette not to foam each sample and reagent to avoid cross contamination between microwells.
- (14) All microwell strips, which are not immediately required, should be returned to the zip lock pouch, which must be carefully resealed to avoid moisture absorption.
- (15) Wash concentrate may become turbid at 2-8 °C, which does not cause inconsistent results.
- (16) Implement used for the test should be disposed or treated as shown below.

Soak in 2% final conc. glutaraldehyde solution for more than 1 hour or soak in 0.5% Sodium hypochlorite solution (available chlorine: approx. 5000ppm) for more than 1 hour or autoclave at 121 °C for more than 20 minutes.
- (17) The anti-RNA Polymerase III antibodies value obtained from this assay are an aid to diagnosis only. Each physician must interpret these results in light of the patient's history, physical findings, and other

diagnostic procedure.

## **MATERIALS REQUIRED BUT NOT PROVIDED**

- Microplate reader (wavelength: 450 nm, 620 nm/reference)
- Multichannel micropipette (e.g. 100 µl - 300 µl) for dispensing conjugate, substrate, and stop solution.
- Single channel pipette (10 µl & 100 µl)
- Reagent reservoir
- Autowasher or wash bottle
- Deionized or distilled water
- One liter graduated cylinder for preparation of wash solution
- Test tubes for patient sample dilutions (e.g. 1000 µl)
- Disposable pipette tips
- Paper towels
- Basin and disinfectant
- Microplate cover

## **PROCEDURE**

### ■ **PREPARATION OF REAGENTS**

- Bring all assay materials to room temperature (20-25 °C) prior to use.
- Microwell Strips: Remove required microwell strip from pouch and place them in the frame. Promptly return unused strips to refrigerated storage.
- Wash Solution: Prepare 1:10 dilution of The Wash Concentrate prior to use. (ex. add 100 ml of Wash Concentrate to 900 ml of distilled water). The diluted wash solution is stable for 2 weeks at 2 -8°C.
- Do not dilute Calibrator 1, Calibrator 2, Positive control, Negative control, Conjugated Reagent, Assay Diluent, Substrate and Stop Solution, which are ready-to-use.

### ■ **PREPARATION OF SAMPLES**

- Use fresh patient sera. If storage is needed, they should be frozen below -20°C for up to one month, below -70°C for longer storage. Do not repeat freezing and thawing.
  - \*In case stored below -20°C for more than 6 months or freezing and thawing repeatedly, nonspecific results are obtained because of IgG denaturation.
- Dilute each patient serum 1:101 by adding 10µl of serum to 1ml of Assay Diluent.
  - \*Diluted samples must be used within a day.
  - \* Assay Diluent may form precipitate, which does not cause inconsistent results.

### ■ **ASSAY PROCEDURE**

#### **STEP 1. (SAMPLE INCUBATION)**

Using the multi-channel pipetor, transfer 100µl of Calibrator 1, Calibrator 2, Positive control, Negative control and each diluted sample into the appropriate microwells of the antigen test plate. Add Calibrators directly to appropriate wells. ( Do not dilute Calibrators and Controls.)

- \* Incubation starts on pipetting to the antigen-coated microwells. Pipetting should be completed as quickly as possible.

Cover wells with a plate sealer and incubate for 60 minutes at room temperature (20-25 °C).

**STEP 2. (WASHING)**

Aspirate or discard the well contents. Fill the well with Wash Solution and then completely aspirate or discard the contents. Wash 4 times. Tap the plate on a paper towel to remove any remaining Wash Solution. When autowasher is used, wash 4 times.

- \* Each laboratory is recommended to confirm its own appropriate washing times and set-up.
- \* Wash Solution should be used at 20-25 °C.

**STEP 3. (CONJUGATE INCUBATION)**

Pour Conjugated Reagent into a reservoir. Add 100 µl of the Conjugated Reagent to each well with multichannel pipette. Cover wells with the plate sealer and incubate for 60 minutes at room temperature (20-25°C).

**STEP 4. (WASHING)**

Wash the microplate following the STEP 2 procedure.

**STEP 5. (SUBSTRATE INCUBATION)**

Pour Substrate into a reservoir and pipette 100 µl of the Substrate to each well with multichannel pipette.

- \* The reservoir should be different from the one, which was used for pouring conjugate solution. A new disposable reservoir should be used because Substrate is easily oxidized by metal ion.
- \* The Substrate, once poured in a reservoir, should not be returned to the bottle.

Cover wells with the plate sealer and incubate for 30 minutes at room temperature (20-25°C).

**STEP 6. (STOP REACTION)**

Pour Stop Solution into a reservoir. Pipette 100 µl of the solution to each well with multichannel pipette.

**■ READING**

Read the absorbance of each well at 450 nm. If a dual wavelength plate reader is available, set the test wavelength at 450 nm and the reference at 620 nm.

- \* Reading should be done as quickly as possible after stopping the reaction.
- \* Ensure that the bottom of the plate is clean and dry, and that no air bubbles are present on the surface of the liquid in the wells before reading the plate.

**■ CALCULATION OF RESULT**

$$\text{Unit value (U/ml)} = \frac{(A_{450}\langle\text{Sample}\rangle - A_{450}\langle\text{Calibrator 1}\rangle)}{(A_{450}\langle\text{Calibrator 2}\rangle - A_{450}\langle\text{Calibrator 1}\rangle)} \times 100$$

\*A450 is abbreviation of absorbance value at 450 nm.

\*An international reference material for anti-RNA Polymerase III antibodies is not available, the assay is calibrated in relative arbitrary units.

**■ QUALITY CONTROL**

The mean absorbance of Calibrator 1 should be  $\leq 0.100$  and Calibrator 2 should be  $\geq 0.500$ . Failure to achieve these values may indicate that the kit is no longer suitable for use. If any of these criteria are not met, the results are invalid and the test should be repeated. Before repeating an assay, check the following procedure steps.

- Incubation Temperature
- Incubation Times

- Washing
- Sample dilutions

### **TEST INTERPRETATION AND EXPECTED VALUE**

The following is intended only as a guide for interpretation. Each laboratory is recommended to establish its own criteria for test interpretation based on sample populations typically encountered.

Anti-RNAP III value (U/ml)	Interpretation
< 28	Negative for Anti-RNAP III Ab
Greater than or equal to 28	Positive for Anti-RNAP III Ab

### **■ LIMITATIONS**

As with other diagnostic test procedures, the results obtained with the anti-RNA Polymerase III ELISA Kit serve only as an aid to diagnosis and should not be interpreted as diagnostics in themselves.

### **PERFORMANCE CHARACTERISTICS**

#### **■ CLINICAL SPECIFICITY AND SENSITIVITY**

Disease	Positive sample	Positive rate
MCTD	0/10	0.0%
SLE	0/20	0.0%
SjS	0/27	0.0%
SSc	14/40	35%
CREST	2/5	40%

MCTD: Mixed connective tissue disease

SLE: Systemic lupus erythematosus

SjS: Sjögren's syndrome

SSc: Systemic sclerosis

Define CREST

#### **■ PRECISION**

Repeatability was demonstrated by testing 3 samples in 8 replicates. Reproducibility was determined by testing 3 samples on 5 different days (day-to-day) and by testing 3 samples in 2 lots (lot-to-lot). %CV values for reproducibility and repeatability were below 15% for each sample.

#### **■ ASSAY RANGE**

The assay range of this kit is from 5U/ml to 300U/ml.

#### **■ INTERFERING SUBSTANCES**

Hemoglobin (up to 523 mg/dl), Bilirubin (up to 20.9mg/dl), chyle (up to 2,800 unit as Formazine) and/or

Rheumatoid factor (up to 500 IU/ml) are not affective on the assay result, but avoid using highly hemolysed samples or highly lipemic samples.

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