



## Anti-U1-snRNP (68 kDa) ELISA Kit

Catalog Number EA-5006

(For Research Use Only)

### Introduction

Mixed connective-tissue disease (MCTD) is an autoimmune disorder with coexistence and overlap of various connective-tissue diseases (CTDs) such as systemic lupus erythematosus (SLE); systemic sclerosis (SSc); dermatomyositis (DM); polymyositis (PM); and, occasionally, Sjögren syndrome. The presence of antibodies to specific components of U1-ribonucleoprotein (U1-RNP) complex is the immunological marker for the diagnosis of MCTD. The complex is composed of U-rich small nuclear RNA and a set of proteins, the 68 kDa (or 70 kDa) U1-specific protein plus proteins A and C and the Sm antigens (B, B', D1, D2, D3, E, F, and G). Antibodies against the snRNP complex are directed against Sm as well as the 68 kDa U1-specific proteins plus proteins A and C. It is now known that the availability of RNP antigen in the absence of Sm is a good marker for MCTD.

### Principle of the assay

Anti-U1-snRNP (68 kDa) ELISA kit measures anti-U1-snRNP antibodies in the serum. It is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes U1-snRNP (68 kDa) protein for immobilization on the microtiter wells and anti-human IgG antibodies conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two components, resulting in anti-U1-snRNP antibodies being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of anti-U1-snRNP (68 kDa) is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

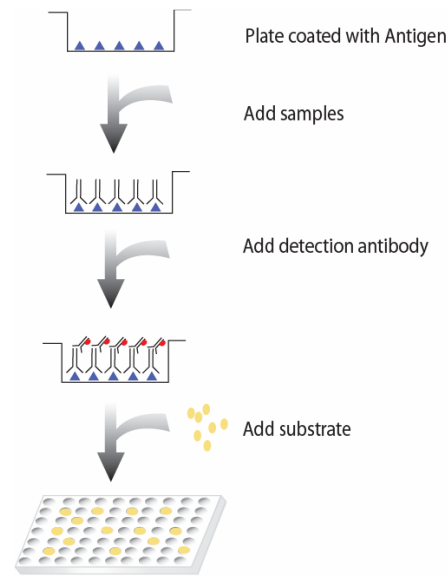


Diagram of ELISA

### Materials provided with the kit

- 8x12 96-well plate coated with U1-snRNP 68 kDa (4°C).
- Anti-human IgG antibody conjugated to HRP (4°C).
- 1X Diluent buffer (4°C).
- 5X Assay wash buffer (4°C).
- Substrate (4°C).
- Stop Solution (4°C)

### Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Shaker

## Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer  
40ml 5x Assay wash buffer  
160ml ddH<sub>2</sub>O
- Dilute 1000 times of anti-mouse IgG antibody conjugated to HRP with 1X Diluent buffer.
- Avoid contact of Substrate and Stop Solution with sunlight or any metal surfaces.
- Important Note: Before beginning your experiment, quickly take 50µL of the Substrate and check to make sure it's clear. **If its color has changed to dark blue, do not begin the experiment and contact us immediately.**

## Storage and Preparation

Store all reagents at 2-8°C.

All reagents must be brought to room temperature (20-25°C) prior to use.

When stored at 2-8°C, the diluted Assay wash buffer is stable until the kit expiration date.

## Precautions

Human blood derivatives and patient specimens should be considered potentially infectious. All human derived components need to be tested for the negative HBsAg, HCV, HIV-1 and 2 and HTLV-I. Follow good laboratory practices in storing, dispensing and disposing of these materials.

## Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Add 100µl of diluent buffer to the wells to be used. Then add 1µl of sample or positive control directly in the well to make a 1:100 dilution. Incubate for 1 hour at room temperature with gentle shaking. \*Note: We recommend having a blank condition. For the blank, add only diluent buffer to the well.
3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process twice for a total of three washes. Completely remove liquid at each wash by firmly tapping the plate against clean paper towels.
4. Add 100µl of diluted anti-human IgG antibody conjugated to HRP to each well and incubate for 0.5 hours at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Before adding substrate, check to make sure it is clear. **If the substrate is already blue, please leave the wash buffer in the wells, seal and store the plate at 4°C, and contact us immediately.**
7. Add 100µl of Substrate to each well and incubate for 5-30 minutes.
8. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
9. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

## Example

