



## Anti-CENP-B ELISA Kit

Catalog Number EA-5008

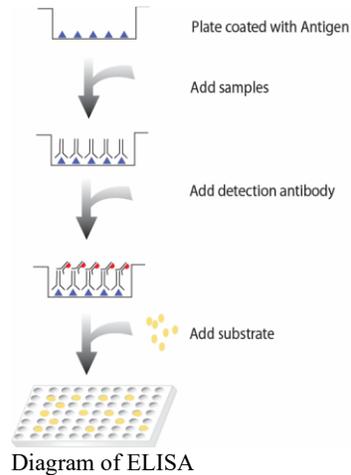
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### Introduction

Anti-centromere antibodies (ACA) are an immunological marker for diagnosis of CREST syndrome, a limited form of systemic sclerosis. At least 9 proteins are known to be associated with the centromere complex, but CENP-B is normally considered to be the major centromere antigen. CENP-B has a molecular weight of approximately 66 kDa and plays an important role in the formation of the centromeric chromatin. CENP-B antibodies are present in the sera of up to 80% of patients with CREST syndrome. These autoantibodies are also often detected in sera from patients with Raynaud's phenomenon and occasionally in other rheumatic diseases such as systemic lupus erythematosus, Sjögren's syndrome, and rheumatoid arthritis. ACA have also been reported to occur with high prevalence in patients with primary biliary cirrhosis, in patients with malignancies and occasionally in normal individuals.

### Principle of the assay

Anti-CENP-B ELISA kit measures anti-CENP-B antibodies in the serum. It is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes CENP-B protein for immobilization on the microtiter wells and anti-mouse IgG antibodies conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two components, resulting in anti-CENP-B 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-CENP-B is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



### Materials provided with the kit

Component	Qty	Store at
<b>8x12 96-well strip Plate coated with CENP-B</b>	1	4°C
<b>Anti-Human IgG antibody conjugated to HRP</b>	10µL	4°C
<b>1X Diluent Buffer</b>	40mL	4°C
<b>5X Assay Wash Buffer</b>	40mL	4°C
<b>Substrate</b>	10mL	4°C
<b>Stop solution</b>	5mL	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-human 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.**

## 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. Take the desired number of well strips from the plate. Make sure the rest of strips are well sealed.
2. Add 100 µl of diluted samples per well and incubate for 1 hour at room temperature with gentle shaking. \*Note: We recommend having a blank condition. For the blank, add only 1X 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 30 minutes 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 7-30 minutes. \*Note: Samples should be stopped when blue color reaches maximum intensity.
9. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.