Signosis
Innovative Plate Assay Solutions

Mouse Anti-CENP-B ELISA Kit

Catalog Number EA-5206 **(For Research Use Only)**

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.

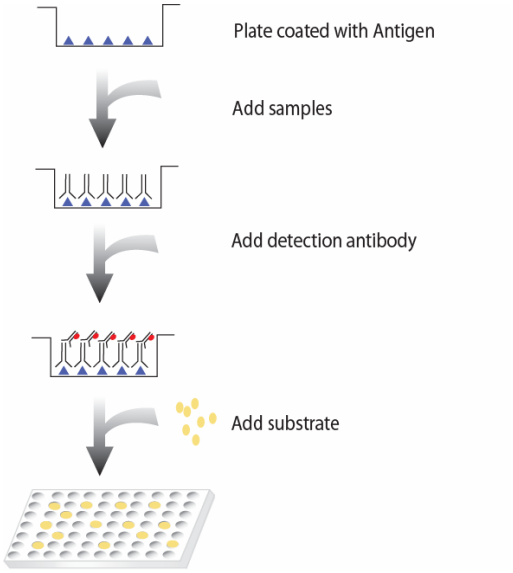

Diagram of ELISA

Materials provided with the kit

- 8x12 96-well plate coated with CENP-B (4°C).
- Anti-mouse 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 ddH2O
- Dilute 1000 times of anti-mouse IgG antibody conjugated to HRP with 1X Diluent buffer.

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.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

Plasma

Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

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 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-mouse 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. Add 100µl of Substrate to each well and incubate for 5-30 minutes.
7. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
8. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.