Version 14-04/24

User's Manual

Mouse Anti-CENP-B ELISA Kit

REF **DEIA118J**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Anti-CENP-B ELISA kit measures anti-CENP-B antibodies in the serum.

General Description

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.

Principles of Testing

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.

Reagents And Materials Provided

1. 8×12 96-well plate coated with CENP-B (4°C).
2. Anti-mouse IgG antibody conjugated to HRP (4°C).
3. Mouse CENP-B Positive Control (4°C).
4. 1× Diluent buffer (4°C).
5. 5× Assay wash buffer (4°C).
6. Substrate (4°C).
7. Stop Solution (4°C)

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm

2. Shaker

Storage

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.

Specimen Collection And Preparation

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.

Reagent Preparation

1. Dilute the 5× Assay wash buffer to 1× buffer 40ml 5× Assay wash buffer 160ml ddH_2O
2. Dilute 1000 times of anti-mouse IgG antibody conjugated to HRP with 1X Diluent buffer.

Assay Procedure

1. Take the desired number of well strips from the plate. Make sure the rest of strips are well sealed.
2. Standard Curve:

   Add 200µl 1×Diluent Buffer to the 1st well on one strip

   Add 100µl 1× Diluent Buffer to the rest of wells on the same strip

   Add appropriate amount of mouse CENP-B positive control (50 µg/ml) to 1st well as 1st dilution

   Mix 1st dilution in 1st well and transfer 100µl from 1st to next well for next dilution. Perform six two-fold serial dilutions

   1×Diluent buffer serves as the zero standard or blank **Note: The first dilution starting from 250ng/ml is recommended.**

3. Add 100 µl of diluted samples or positive control (1:100 diluted with 1× Diluent Buffer) 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 1× Diluent buffer to the well.**

4. Aspirate each well and wash by adding 200µl of 1×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.

5. Add 100µl of diluted anti-mouse IgG antibody conjugated to HRP to each well and incubate for 30 minutes at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 3.
7. Add 100µl of Substrate to each well and incubate for 5-15 minutes.***Note: Positive control will turn blue. Samples should be stopped when blue color begins to appear in blank.**
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.

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