



Mouse Anti-Histone ELISA Kit

Catalog Number EA-5208

(For Research Use Only)

Introduction

Anti-histone antibody is an important immunological marker for the diagnosis of drug induced lupus (DIL). Although anti-histone antibodies are detected in approximately 30% to 60% of patients with systemic lupus erythematosus (SLE), their presence is much higher in DIL patients. They are detected in about 95% of DIL patients, which is more important diagnostically. DIL is clinically similar to SLE, except that the former patients do not have kidney or central nervous system involvement. In addition, DIL patients do not demonstrate the multiple types of antinuclear antibodies (ANAs) often found in SLE patients.

Principle of the assay

Anti-histone ELISA kit measures anti-histone antibodies in the serum. It is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes histone 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-histone 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-histone is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

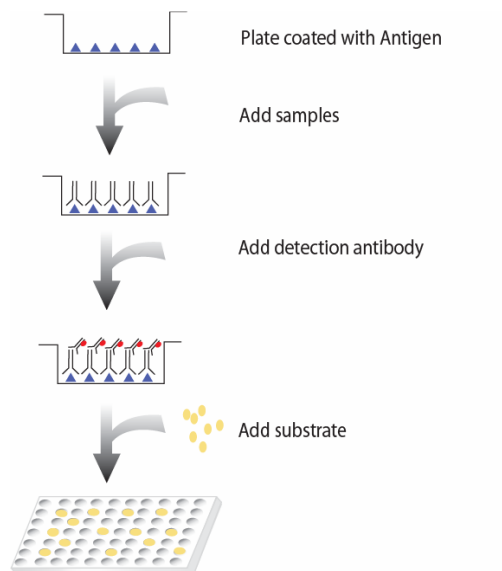


Diagram of ELISA

Materials provided with the kit

- 8x12 96-well plate coated with histone (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.
- Avoid contact of Substrate and Stop Solution with 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.

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. 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 (1:100 diluted with 1X 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 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-mouse 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 5-15 minutes.
*Note: Samples should be stopped when blue color begins to appear in blank/negative control.
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.