Signosis
BioSignal Capture

Mouse Anti-dsDNA ELISA

Catalog Number EA-5201 **(For Research Use Only)**

Introduction

Anti-dsDNA antibodies that appear to be critical in the pathogenesis of tissue injury are characteristic of systemic lupus erythematosus (SLE). There is a good correlation between anti-dsDNA antibody levels and disease activity. The overall detection rate of these antibodies is approximately 50-55% in SLE patients and about 89% in SLE patients with active renal disease. When they are present in high concentration, anti-dsDNA antibodies are virtually specific for SLE (>90%). Antibodies to dsDNA may disappear with immunosuppressive treatment and during remission. They rarely occur in other autoimmune disorders. Signosis has developed anti-dsDNA ELISA, a sandwich quantitative assay, to screen the presence of serum ds-DNA antibodies IgG.

Principle of the assay

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

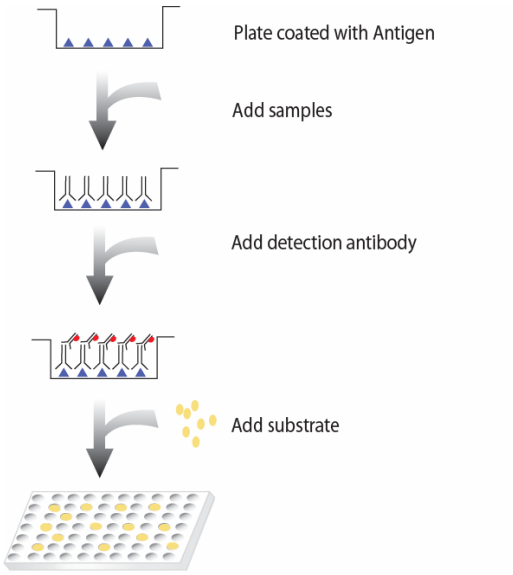


Diagram of ELISA

Materials provided with the kit

Component	Qty	Store at
96-Well 12 strip Plate coated with ds-DNA	1	4°C
Anti-mouse IgG conjugated to HRP	10µL	4°C
dsDNA mouse IgG standard 25 µg/ml	10µL	4°C
1X Diluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	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 ddH2O
- Dilute 1:1000 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.

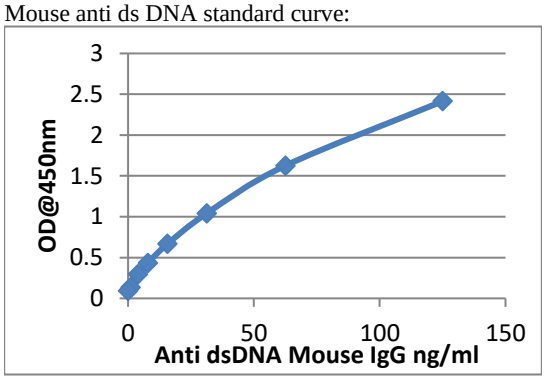
Assay procedure

1. Calculate the number of samples to decide how many strips need to be used. Make sure the rest wells are well sealed with the seal provided.
2. Standard Preparation:
   - Add 200µl 1X Diluent Buffer to the 1st well on one strip
   - Add 100µl 1X Diluent Buffer to the rest of wells on the same strip
   - Add appropriate amount of dsDNA mouse IgG standard (25 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
   - 1X Diluent buffer serves as the zero standard or blank

Note: The first dilution starting from 125ng/ml is recommended.

3. Add 100µl of 1X 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
4. 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.
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 4.
7. Add 100µl of Substrate to each well and incubate for 7-30 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 of samples should change from blue to yellow.
8. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Mouse anti ds DNA standard curve:



This Standard curve is for demonstrative purpose only.

A standard curve must be run with each assay.

Assay range: 4 ng/ml to 125 ng/ml
Sensitivity: 0.5ng/ml