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.

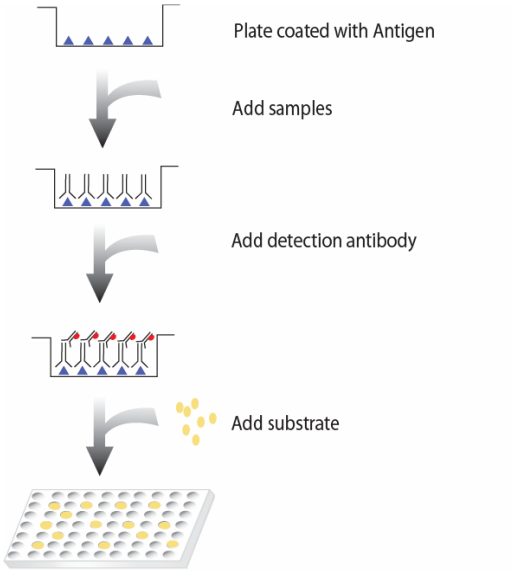



Diagram of ELISA

## 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.
- 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.**

## 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.

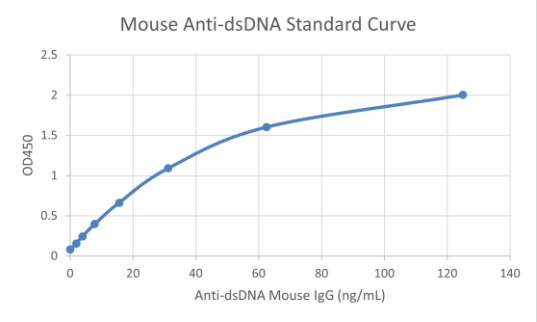
## Assay procedure

1. Calculate the number of samples to decide how many strips need to be used. Make sure the rest of the wells are well sealed.
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 1µl 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. 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.**
8. 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.**
9. Add 50µl of Stop solution to each well. The color of samples should change from blue to yellow.
10. 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 can be run with each assay.

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