Signosis
Innovative Plate Assay Solutions

Human Anti-dsDNA ELISA Kit

Catalog Number EA-5002 **(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.

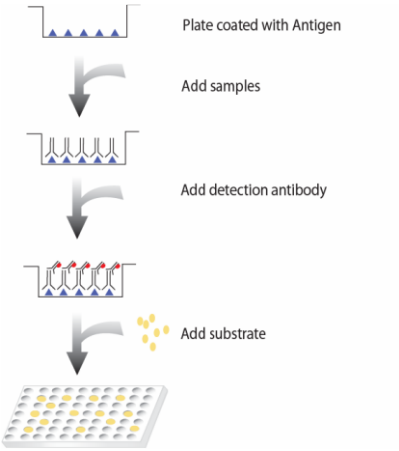

Diagram of ELISA

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.

Materials provided with the kit

Component	Qty	Store at
8x12 96-well strip Plate coated with ds-DNA	1	4°C
Anti-Human IgG antibody conjugated to HRP	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 1000 times of anti-human 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.

Precautions

Human blood derivatives and patient specimens should be considered potentially infectious. All human derived components need to be tested for the negative HBsAg, HCV, HIV-1 and 2 and HTLV-I. Follow good laboratory practices in storing, dispensing and disposing of these materials.

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 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-Human 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 7-30 minutes. *Note: 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.