# Quantitative Enzyme-linked Immunosorbent Assay (ELISA) to Measure Serum Levels of Murine Anti-dsDNA Antibodies (1)

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**[Abstract]** ELISA is an easy and relatively sensitive way to measure protein concentration. This protocol describes how to measure serum levels of murine anti-dsDNA antibodies. Circulating anti-dsDNA antibody is a hallmark of SLE both in human patients and in many SLE mouse models. This protocol was developed or modified in Dr. Anne Davidson's lab at Feinstein Institute for Medical Research.

## Materials and Reagents

1. Salmon sperm DNA (Life Technologies, Invitrogen™, catalog number: AM9680)
2. Murine immunoglobulin of the desired isotype (Sigma- Aldrich, catalog number: M5284)
3. Horseradish peroxidase (HRP) conjugated goat anti-mouse isotype specific antibodies (Southern Biotech)
4. 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) Peroxidase Substrate Solution A (Kirkegaard & Perry Laboratories, catalog number: 50-64-00)
5. ABTS Peroxidase Substrate Solution B (Kirkegaard & Perry Laboratories, catalog number: 50-65-00)
6. ABTS Peroxidase Stop Solution (Kirkegaard & Perry Laboratories, catalog number: 50-85-01)
7. 10x PBS-Tween 20 (see Recipes)
8. Blocking solution (see Recipes)

## Equipment

1. 45-μm syringe top filter (USA Scientific)
2. Clear 96-well Microtest polystyrene assay plate (BD Biosciences)

## Procedure

1. Make double stranded salmon sperm DNA by passage though a 45 μm filter.
2. Add 100 μl/well of 100 μg/ml salmon sperm DNA to a 96-well Microtest assay plate.
3. Wrap the plate with plastic wrap and incubate at 4 °C for overnight.
4. Discard the coating antibody solution and wash the plate with 1x PBS-Tween 6 times.
5. Dry the plate and add 100 μl of blocking solution per well to the plate.
6. Incubate the plate at room temperature (RT) for 1.5 h.
7. Discard the blocking solution and wash the plate with 1x PBS-Tween 5 times.
8. Dilute the mouse serum in 1% BSA in PBS.
9. Add 100 μl/well of diluted serum in duplicates or triplicate to the plate. Serum should be diluted in 1% BSA in PBS and titration is required to achieve optimal detection.
10. Make serial dilutions of a high titer serum sample and add the serial dilution to the plate.
11. Incubate the plate at 37 °C for 1 h.
12. Discard the diluted serum and wash the plate with 1x PBS-Tween 10 times.
13. Add 100 μl/well of HRP conjugated goat anti-mouse isotype specific antibodies (1/4,000 in PBS/1% BSA) to the plate and incubate at 37 °C for 1 h.
14. Discard the diluted serum and wash the plate 10 times.
15. Add 100 μl/well of 1:1 mix of ABTS Peroxidase Substrate Solution A and B to the plate.
16. Develop the plate at RT in dark. Incubation times will vary depending on your assay.
17. Stop the reaction by adding 100 μl/well of ABTS Peroxidase Stop Solution.
18. Read the plate using an ELISA reader with a wavelength of 410 nm.
19. Calculate the concentration of the serum samples using the standard curve established with the serial dilutions of the high titer serum sample.

## Recipes

1. 10x PBS-Tween 20 [0.1 M PBS, 0.5% Tween 20 (pH 7.4)]

   | | |
   |---|---|
   | $Na_2HPO_4$ (anhydrous) | 10.9 g |
   | $NaH_2PO_4$ (anhydrous) | 3.2 g |
   | NaCl | 90 g |
   | Distilled water | 1,000 ml |

   Mix to dissolve and adjust pH to 7.4 and then add 5 ml of Tween 20, store this solution at RT.

   Dilute 1:10 with distilled water before use and adjust pH if necessary
2. Blocking solution

   5% FBS and 3% BSA in PBS

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**References**

1. Mihara, M., Tan, I., Chuzhin, Y., Reddy, B., Budhai, L., Holzer, A., Gu, Y. and Davidson, A. (2000). CTLA4Ig inhibits T cell-dependent B-cell maturation in murine systemic lupus erythematosus. *J Clin Invest* 106(1): 91-101.

 