# Quantitative Enzyme-Linked Immunosorbent Assay (ELISA) to Measure Serum Levels of Murine Anti-histone Antibodies [1]

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**[Abstract]** Autoimmune disease is characterized with the break of tolerance against self antigens. Anti-histone antibodies can be found in the majority of patients with system lupus erythematosus (SLE) and a number of lupus-prone mouse strains. This protocol describes a reliable method to determine the relative serum titers of anti-histone antibodies in these mice.

## Materials and Reagents

1. Histone from calf thymus (Roche Diagnostics, catalog number: 10223565001)
2. Phosphate buffered saline (PBS)
3. Tween 20
4. $Na_2HPO_4$ (anhydrous)
5. $NaH_2PO_4$ (anhydrous)
6. NaCl
7. Fetal bovine serum (FBS) (Hyclone)
8. Bovine serum albumin (BSA)
9. Horseradish peroxidase (HRP) conjugated goat anti-mouse isotype specific antibodies [Southern Biotech, catalog number: 1040-05 (IgA); 1030-05 (IgG); 1021-05 (IgM)]
10. ABTS Peroxidase Substrate Solution A and B (Kirkegaard & Perry Laboratories, catalog number: 50-62-01)
11. ABTS Peroxidase Stop Solution (Kirkegaard & Perry Laboratories, catalog number: 50-85-01)
12. 10x PBS-Tween 20 (see Recipes)
13. Blocking solution (see Recipes)

## Equipment

1. Standard bench-top centrifuge
2. Falcon 96-well ELISA plates (BD Biosciences, catalog number: 35-3915)
3. ELISA reader

4. Parafilm

## Procedure

1. Add 100 µl/well of 10 µg/ml histone in PBS to a Falcon plate.
2. Seal the plate with Parafilm and incubate at 4 °C overnight.
3. Discard histone solution and wash the plate 5 times with 1x PBS-Tween. Dry the plate on paper towel.
   *Note: Washes can be done with an ELISA plate washer or by manually pipeting in and out 1x PBS-Tween.*
4. Add 100 µl of blocking solution per well and block the plate at room temperature (RT) for 90 min.
5. Discard the blocking solution and wash the plate four times with 1x PBS-Tween 10 times.
6. Dilute the mouse serum in 1% BSA in PBS and add 100 µl/well in duplicates or triplicates to the plate. Titration is recommended to determine the optimal dilution.
7. Make serial dilutions of a high titer serum sample and add the serial dilution to the plate.
8. Incubate the plate at 37 °C for 1 h.
9. Discard the diluted serum and wash the plate with 1x PBS-Tween 10 times.
10. Add 100 µl/well of HRP conjugated goat anti-mouse isotype specific antibodies (1/4,000 in 1% BSA/PBS) to the plate and incubate at 37 °C for 1 h.
11. Wash the plate with 1x PBS-Tween 10 times.
12. Add 100 µl/well of 1:1 mix of ABTS Peroxidase Substrate Solution A and B to the plate.
13. Develop the plate at RT in dark. Incubation times will vary depending on your assay.
14. Stop the reaction by adding 100 µl/well of ABTS Peroxidase Stop Solution.
    *Note: The plate needs to be read within 30 min once the reaction is stopped.*
15. Read the plate using an ELISA reader with a wavelength of 410 nm.
16. 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

## Acknowledgments

This protocol was developed or modified in Dr. Anne Davidson's lab at Feinstein Institute for Medical Research, NY, USA. This work was supported by grants from the NY SLE Foundation (RB), Rheuminations, NIH AI082037 and AR 049938-01, NIH (PO1 AI51392 and the Flow Cytometry and Protein Expression and Tetramer Cores of PO1 AI51392).

## References

1. Martin, D. A., Zhang, K., Kenkel, J., Hughes, G., Clark, E., Davidson, A. and Elkon, K. B. (2007). Autoimmunity stimulated by adoptively transferred dendritic cells is initiated by both alphabeta and gammadelta T cells but does not require MyD88 signaling. *J Immunol* 179(9): 5819-5828.

 