

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

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**[Abstract]** The circulating anticardiolipin antibody is a hallmark of antiphospholipid syndrome. It also appears in a number of autoimmune mouse models and is indicative of the break of tolerance against self antigens. This protocol describes a reliable method to determine the relative serum titer of anticardiolipin in autoimmune mouse models.

### Materials and Reagents

1. Cardiolipin (Sigma-Aldrich, catalog number: C0563)
2. Ethanol
3. Phosphate buffered saline (PBS)
4. Tween 20
5. Na<sub>2</sub>HPO<sub>4</sub> (anhydrous)
6. NaH<sub>2</sub>PO<sub>4</sub> (anhydrous)
7. NaCl
8. Fetal bovine serum (FBS) (Hyclone)
9. Bovine serum albumin (BSA)
10. Horseradish peroxidase (HRP) conjugated goat anti-mouse isotype specific antibodies [Southern Biotech, catalog number: 1040-05 (IgA); 1030-05 (IgG); 1021-05 (IgM)]
11. ABTS Peroxidase Substrate Solution A and B (Kirkegaard & Perry Laboratories, catalog number: 50-62-01)
12. ABTS Peroxidase Stop Solution (Kirkegaard & Perry Laboratories, catalog number: 50-85-01)
13. 10x PBS-Tween 20 (see Recipes)
14. Blocking solution (see Recipes)

### Equipment

1. Standard bench-top centrifuge

2. Immulon 2HB plates (Fisher Scientific, catalog number: 14-245-61)
3. ELISA reader

### Procedure

1. Add 100  $\mu$ l/well of 75  $\mu$ g/ml cardiolipin in ethanol to an Immulon 2HB plate and allow it to dry at room temperature.
2. Add 100  $\mu$ l of blocking solution per well and block the plate at room temperature (RT) for 90 min.  
*Note: FBS is the source of beta glycoprotein I.*
3. Discard the blocking solution and wash the plate four times with 120  $\mu$ l/well PBS.  
*Note: Washes can be done with an ELISA plate washer or by manually pipeting in and out PBS.*
4. Dilute the mouse serum in 1% BSA in PBS and add 100  $\mu$ l/well in duplicates or triplicates to the plate.  
*Note: A 1:500 dilution generally gave us optimal results of serum levels of anti-cardiolipin in 12-22 week old male and female NZW x BXSB F1 mice. Titration is recommended to achieve optimal detection.*
5. Make serial dilutions of a high titer serum sample and add the serial dilution to the plate.
6. Incubate the plate at 37 °C for 2 h.
7. Discard the diluted serum and wash the plate with 1x PBS-Tween 10 times.
8. Add 100  $\mu$ l/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.
9. Wash the plate with 1x PBS-Tween 10 times.
10. Add 100  $\mu$ l/well of 1:1 mix of ABTS Peroxidase Substrate Solution A and B to the plate.
11. Develop the plate at RT in dark. Incubation times will vary depending on your assay.
12. Stop the reaction by adding 100  $\mu$ l/well of ABTS Peroxidase Stop Solution.  
*Note: The plate needs to be read within 30 min once the reaction is stopped.*
13. Read the plate using an ELISA reader with a wavelength of 410 nm.
14. 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)]  
 $\text{Na}_2\text{HPO}_4$  (anhydrous)      10.9 g

NaH <sub>2</sub> PO <sub>4</sub> (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. Kahn, P., Ramanujam, M., Bethunaickan, R., Huang, W., Tao, H., Madaio, M. P., Factor, S. M. and Davidson, A. (2008). [Prevention of murine antiphospholipid syndrome by BAFF blockade](#). *Arthritis Rheum* 58(9): 2824-2834.