Using ChonBlock™ for Anti-Antigen Antibody ELISAs

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1. **Coat Plates with Antigen:** Dissolve antigen at 5 - 10 µg/ml in PBS with 0.05% Azide. Add 100 µl of the antigen solution to ELISA plates and incubate at 4°C overnight. Wash the antigen-coated plates with endotoxin-free distilled water 3 times using a wash bottle with manifold. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

2. **Add Blocking Buffer:** Add 100 µl of the ChonBlock™ Blocking/Sample Dilution Buffer to each well and incubate for 1 hour at room temperature.

3. **Prepare Sample Dilutions:** Dilute 10 µl of serum sample with 990 µl of ChonBlock™ Blocking/Sample Dilution Buffer (1:100 dilution) and keep it as a stock solution for future assays. Then, further dilute the sample with ChonBlock™ Blocking/Sample Dilution Buffer depending on the antibody levels. For example, take 200 µl of the sample stock solution and mix with 200 µl of ChonBlock™ Blocking/Sample Dilution Buffer to make a 1:200 dilution.

   NOTE: Chondrex, Inc. recommends running a preliminary assay using various dilutions of sera (1:200, 1:1,000, 1:5,000) in order to determine the optimal dilution of the samples, especially before assaying a large number of samples.

4. **Wash**: Wash the plate with a wash buffer (PBS with 0.05% Tween 20) at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

5. **Add Samples**: Add 100 µl of ChonBlock™ Blocking/Sample Dilution Buffer (blank) and diluted samples to wells in duplicate according to the desired layout. Incubate at room temperature for 2 hours.

6. **Wash**: Wash the plate with the wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Detection Antibody**: Dilute appropriate peroxidase-conjugated detection antibodies in 10 ml of ChonBlock™ Detection Antibody Dilution Buffer. Add 100 µl of the detection antibody solution to each well and incubate at room temperature for 1 hour.

   NOTE: Chondrex, Inc. recommends running preliminary assays using a positive control to optimize the detection antibody concentration.

8. **Wash**: Wash the plate with the wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **Add TMB**: Prepare TMB solution just prior to use. Add 100 µl of the TMB solution to all wells immediately after washing the plate and incubate at room temperature for 10 - 30 minutes.

10. **Stop**: Add 50 ml of 2N sulfuric acid (Stop Solution) to each well.

11. **Read Plate**: Read the OD values at 450 nm (a 630 nm filter can be used as a reference).

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