

ELISA: (Bile anti-*Clostridium perfringens* IgA)

1. Coat Flat-bottomed high binding 96 well plates (Greiner Bio-one) with 100µL CP antigen (10µg/ml) in 0.1M carbonate buffer (PH 9.6) incubate overnight at 4°C.
2. Wash the plates 3x with PBS-Tween 20 (0.05% Tween 20 in PBS, PH 7.4). Place on shaker 5 min each wash.
3. Blocking: Add 200µl of 8% non-fat dry milk in PBS-Tween 20/well for 90 min at 37°C on the shaker.
4. Wash the plates 3x with PBS-Tween 20. Place on shaker 5 min each wash.
5. Dilute bile samples 1:800 in 8% non-fat dry milk in PBS-T, add 100 µl/well (duplicate). Incubate for 90 min at 37°C on an orbital shaker at 100-150 rpm.
6. Wash the plates 3x with PBS-T. Place on shaker 5 min each wash.
7. Add HRP labeled anti-chicken IgA (Novus biologicals) 1:100,000 in 5% non-fat dry milk in PBS-T 100µl/well. Incubate for 30 min at 37°C on an orbital shaker at 150 rpm.
8. Wash the plates 3x with PBS-T. Place on shaker 5 min each wash.
9. Add substrate 3, 3', 5, 5'-tetramethylbenzidine (TMB) 100µl/well and incubate for 10min.
10. Stop the reaction by 1N HCL (100µl/well).
11. Read the optical density at 450nm using microplate ELISA reader.
12. Report IgA values as mean optical density.

0.1M Carbonate buffer pH 9.5:

Na₂CO₃ · 10H₂O, M.W. 286.2 0.859

NaHCO₃, M.W. 84.00.588

Add to nano pure water and bring the total volume to 100 ml.

1X PBS-Tween pH 7.4:

For 1 liter:

NaCl 8.00g

KH₂PO₄ 0.20g

Na₂HPO₄ 1.09g

KCL 0.20g

Adjust the pH to 7.4 and add Tween-20 to 0.5 ml.

TMB 10mg/ml: In DMSO store at 4° C

Working TMB stock:

(0.1M Sodium Acetate 0.333ml of 3M Sodium Acetate+9.667ml Water)

100ul of TMB (10mg/ml) + 9.9ml 0.1M Sod.Acetate+3ul H₂O₂

3M Sodium Acetate PH 5.2

Dissolve either 40.8 g sodium acetate (m.w. 136.08) trihydrate or 24.6g sodium acetate anhydrous in 70 ml of deionized or distilled water. Once all the salts dissolved transfer to fume hood and adjust the pH to 5.2 by adding glacial acetic acid; monitor with a pH meter. Add water to bring the total volume of solution to 100 ml. Autoclave the bottle and store at room temperature.