**Wild Bird IgY ELISA**

1. COATING. Coat wells of a flat-bottomed 96-well plates with 100 µl/well of sample serum, diluted 1:100 in coating buffer. Shake and verify well bottom.

**INCUBATE overnight at 37C.**

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1. Remove coating solution
2. BLOCK Add 200ul Blocking buffer to each well. Shake and verify well bottom.

**INCUBATE for 30min at room temperature.**

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1. WASH wells 4x with wash buffer
2. 50ul of goat anti-bird IgG (Bethyl A140-110P) diluted 1:1000 in blocking buffer

**INCUBATE for 1 hour at 37C**

\***35 minutes prior to completion of step #5 incubation**, warm ABTS to room temperature in dark place; switch on plate reader.

1. WASH wells 4x with wash buffer
2. DETECTION. Add 100µl/well of ABTS substrate to all wells. **Incubate in the dark at room temperature for 5 minutes.**
3. STOPPING. Add 100ul/well of 1% SDS to all wells (NO BUBBLES!). Swirl to mix. Read plate at 405 nm.