Ketterson / Nolan Research Group Collection

This document is part of a collection that serves two purposes. First it is a public archive for data and documents resulting from evolutionary, ecological, and behavioral research conducted by the Ketterson-Nolan research group. The focus of the research is an abundant North American songbird, the dark-eyed junco, *Junco hyemalis*, and the primary sources of support have been the National Science Foundation and Indiana University. The research was conducted in collaboration with numerous colleagues and students, and the objective of this site is to preserve not only the published products of the research, but also to document the organization and people that led to the published findings. Second it is a repository for the works of Val Nolan Jr., who studied songbirds in addition to the junco: in particular the prairie warbler, *Dendroica discolor*. This site was originally compiled and organized by Eric Snajdr, Nicole Gerlach, and Ellen Ketterson.

Context Statement
This document was generated as part of a long-term biological research project on a songbird, the dark-eyed junco, conducted by the Ketterson/Nolan research group at Indiana University. For more information, please see IUScholarWorks (https://scholarworks.iu.edu/dspace/handle/2022/7911).

IgG ELISA
Prepared by Jen Grindstaff

Purpose: This ELISA gives a quantitative measure of total IgG levels in plasma from Japanese quail.

Protocol:

Day 1

1. Prepare carbonate-bicarbonate coating buffer (0.1 M, pH 9.6) for binding the capture antibody to the microplates.

   Make enough so that there are 11 mL of coating buffer for each plate you want to coat

2. Dilute the capture antibody (donkey, anti-chicken [Jackson ImmunoResearch]) to a concentration of 3 μg/mL in the coating buffer. Add 100 μl of the coating buffer-capture antibody solution to each well of the plate. Seal the plate with plate sealing tape and incubate the plates overnight at 4° C.

Amount of coating buffer:

100 μl/well * 100 wells/plate * # of plates=_____ (+ extra)

Amount of donkey anti-chicken IgG:

(3 μg/ml * volume of coating buffer)/concentration of antibody solution=_____

Day 2

1. Wash the plates using wash buffer just before you start working with them.

Turn on plate washer.
Select Run
Method = Demas
Last Strip =12

Clean Plate Washer using prime/rinse option (immediately after use) and turn off.
Make sure you are rinsing with distilled water.

2. Post coat (Blocking): Add 200 μl 5% milk powder (MP) diluted in 0.01 M PBS to each well.

3. Incubate >2 hours at room temperature.

4. Wash plates with PBS/Tween 20

5. Serial dilution of test serum. Test serum is added to the wells in duplicate/sample. Diluent used is PBS/Tween 20 1%MP for all sera and antibodies.

   Prepare 1:20,000 dilutions of the samples in PBS-T/1% MP and then add 100 μl of the diluted samples to the plates in duplicate.

   Blanks: Use 2-6 wells as blanks on each plate. Add 100 μl PBS/1% MP to these wells.

6. Prepare a serial dilution of the chicken-IgG standard [Promega Chicken IgY] for a standard curve (0.1 μg/ml, 0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.00156, two samples per concentration). Use PBS-T/1% MP for the dilutions. Add the standards to the plates in duplicate (2 x 100 μl).

7. Seal the plates with plate sealing tape and incubate the plates overnight at 4 C.

Day 3

1. Wash the plates using the procedure on day 2.

2. Prepare a 1:1000 dilution of the labelling antibody (AP-conjugated rabbit, anti-chicken [Sigma]) in PBS-T/1% MP and add 100 μl of the solution to each well (make up 12 mL of solution for each plate). Seal the plates with plate sealing tape and incubate them for 1 hour at 37 C.

3. Prepare the diethanolamine-pNPP substrate buffer for visualization. Use automatic pipetter for diethanolamine.

   pH to 9.8, than add dH_2O to get up to appropriate volume

   Leave buffer stirring, and right before incubation is up, add the *light sensitive* substrate (stored in -20°C freezer).

4. Wash plates with PBS/Tween. Blot plates *thoroughly* or enzyme-substrate reaction may be inhibited.

5. Add 100 μl of the buffer to each well of the microplates. Read the plates immediately at 405 nm. Read plate at intervals of 30 seconds beginning immediately and continuing for 14 minutes using a kinetic protocol.

Reagents:

PBS Wash Buffer (0.05 M, pH = 7.4)

For 1 L

1 Add: 950 ml dH_2O
1.67 g sodium phosphate-dibasic (Na_2HPO_4)
0.57 g sodium phosphate-monobasic (NaH_2PO_4)
8.5 g sodium chloride (NaCl)
10 mg sodium azide (NaN_3)

2. q.s. to 1 L and pH to 7.4

3. Add 0.5 ml Tween-20

For 4 L

1. Add: 3.8 L dH20
6.68 g Na_2HPO_4
2.28 g NaH_2PO_4
34.0 g NaCl
40 mg NaN3

Substrate Buffer

For 50 ml

1. Add: 40 ml dH_2O
4.85 ml diethanolamine
0.01 g sodium azide

2. pH to 9.8 and q.s. to 50 ml

3. Add 1mg/ml PnPP (add when ready to use)

4. Store protected from light

Carbonate-Bicarbonate Coating Buffer (0.1 M, pH = 9.6)

For 1 L

1. Add: 750 ml dH2O
4.24 g Na_2CO_3
5.04 g $NaHCO_3$

2. q.s. to 1 L and pH to 9.6

*Make enough for 12 ml per plate