

HORMONE ASSAYS

September 26: Testosterone EIA Lab

We will meet in the Ketterson Lab, JH 033.

Plate(s) will be loaded with samples, standard curve, and reagents ahead of time, because of the 2 hour (or overnight) incubation step. The incubation will take place overnight before the class. These steps will be explained thoroughly before proceeding with the assay. *Radiolabeled testosterone will not be used in these assays, as students may not be trained in radioactivity safety. We will discuss the method of using radioactively labeled testosterone to measure recovery efficiency.*

Steps:

1. Use plate washer to empty & wash out wells.
2. Add 5µl Blue Conjugate to 2 TA wells.
3. Add 200 µl p-Npp substrate solution to every well.
4. Incubate plate for 1 hour at 37°C.
5. Add 50µl Stop Solution to every well and read plate on plate reader.

We only have enough samples to do one EIA plate; however, all students will practice the methods (including setting up a standard curve) with practice plates.

We will discuss how to calculate and interpret results from the EIA, and fill out the EIA worksheet (available on protocol website).

Additional Demonstration: Egg yolk extraction [during incubation hour]

To save time, only the preparation of yolk samples will be done with the class.

Steps:

1. Separate yolk from the rest of the frozen egg.
2. Homogenize yolk
3. Add 5-15 mg of yolk to 1.5 ml Eppendorf tube
4. Add 500 µl distilled water and vortex until homogenized (use 2-3 glass beads).

After this, extraction of steroids is very similar to other methods.

READING LIST

Clotfelter et al. 2004. Consequences of elevating plasma testosterone in females of a socially monogamous songbird: evidence of constraints on male evolution? *Hormones and Behavior* 46: 171-178. *Uses EIA with both plasma and egg yolk.*

Wingfield and Farner 1975. The determination of five steroids in avian plasma by radioimmunoassay and competitive protein-binding. *Steroids* 26: 311-327.