**Solid phase extraction (SPE) for extracting CORT from egg yolk - updated 28/04/2023 by OC**

1. Thaw yolk samples at room temperature. Once thawed, samples should NOT sit for more than 30 minutes. For this reason, it is best to thaw ~4 at a time.
2. Prepare the clean silica bonded C18 vacuum columns by adding 10mL of dd H2O (5mL + /5mL) and drawing through the liquid slowly. Do not allow columns to run dry (turn off spigot with 1mm meniscus remaining above column substrate).
3. Add diluted samples to each individual prepared column using a P1000 pipette. Add 1mL ddH20 to the tube, vortex, and add this rinse to the column as well.
4. Draw the samples slowly through individual columns. Steroids should now be bound to the substrate of the column with strong polar bonds.
5. Wash each column with 5mL 40% methanol to remove lipids (weak polar bonds in 40% methanol, will wash out lipids, but not disrupt the strong polar bonds between the steroids and column substrate). Do not allow columns to run dry (turn off spigot with 1mm meniscus remaining above column substrate). Discard the flow through liquid.
6. Add 5mL 100% methanol solution to each column, allow to soak for 2 minutes. Elute the columns into a glass collection tube (this flow through liquid contains the steroids of interest). Allow column to run dry (open spigot to maximum for 2 mins to draw through all liquid).
7. Dry the tubes under nitrogen at 37 ºC, until fully evaporated.
8. Cap and store the sample tubes at -20 ºC.

**Extraction efficiency (EE) must be measured for each lot of samples that is extracted.**

1. Combine 2-3 thawed yolk samples that are set aside for this purpose (i.e., do NOT use samples from your experiment).
2. Vortex and centrifuge samples from 3 mins at 5000 rpm.
3. Remove supernatant and put in a new Eppendorf tube.
4. Aliquot 2 x 500ul of sample into two new Eppendorf tubes (EE and ES).
5. Spike one aliquot (ES) with 10ul of the CORT standard supplied with the CORT EIA kit and vortex (this is equivalent to a 1000 pg spike in CORT).
6. Extract each sample as per above EXCEPT use 500ul of ddH20 to rinse samples instead of 1mL of ddH20.

**Extraction efficiency – spiking samples day of EIA.**

1. Reconstitute EE and ES samples using methods for reconstituting samples.
2. Determine extraction efficiency percentage as: [(ES – EE)/spike] \* 100