**Solid phase extraction (SPE) for extracting estradiol from serum - 26/06/2023 by OC**

1. Thaw serum 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. Dilute serum with assay buffer from EIA kit to desired dilution. Naomi: can you add which dilution we decided to use here?
3. 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 pump with 1mm meniscus remaining above column substrate).
4. Add diluted samples to each individual prepared column using a P1000 pipette. Add 500 ul assay buffer to the tube and add this rinse to the column as well.
5. Draw the samples slowly through individual columns. Steroids should now be bound to the substrate of the column with strong polar bonds.
6. 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 pump with 1mm meniscus remaining above column substrate). Discard the flow through liquid.
7. 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 (maximize pressure for the last 30 seconds to draw through all liquid).
8. Dry the tubes under nitrogen at 35 ºC, until fully evaporated.
9. Cap and store the sample tubes at -20 ºC.

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

1. Combine serum samples that are set aside for this purpose (i.e., do NOT use samples from your experiment) or used a designated pooled sample.
2. Make two 500ul aliquots from a pooled sample.
3. Spike one aliquot with 6ul of the estradiol standard supplied with the EIA kit and vortex.
4. Extract each sample as per above.
5. Calculate extraction efficiency as: (spiked – not spiked) / concentration of spike.