Making ECGS

Materials:
0.15 M NaCl (500 ml)
370 g Bovine hypothalamus (Pel-Freez Catalog number 57117-2)
40% w/v Streptomycin Sulfate (Fisher catalog number BP910-50)
0.45 and 0.22 micron filtration units (500 ml capacity, Corning Catalog numbers 430770 and 430769)

Day 1:

Prepare 500 ml of 0.15 M NaCl per batch of ECGS (370 g hypothalamus). Store at 4C overnight.

Day 2:

Using a blender, combine 370 g bovine hypothalamus (-80C Pel-Freez Catalog 57117-2) and 500 ml 0.15 M NaCl solution.

Homogenize hypothalamus until a smooth liquid. Approximately 20 minutes of non-stop blending is needed. CRITICAL STEP! UNDER BLENDING = LOW PROTEIN CONCENTRATION AT END

Stir for 2 hours at 4C (Prepare 3-5 ml 40% Streptomycin sulfate in milliQ water while waiting).

Centrifuge at 13,700 g for 40 min at 4C. Use a serological pipette to remove supernatant (red liquid). Discard fatty portion.

Add streptomycin sulfate (40%) solution until diluted to 0.4% in supernatant
(1 ml streptomycin = 100 ml extract)

Incubate on ice, at 4C, for 2 hours.

Centrifuge at 13,700 g for 40 min at 4C. Use a serological pipette to remove supernatant (red liquid). Discard fatty pellet.

Pre-filter supernatant through 0.45 micron filter (500 ml bottle filters). Keep on ice.

Sterile filter through 0.22 micron filter (500 ml bottle filters). Keep at 4C until aliquoting.

Day 3:

Determine protein content of ECGS using the Bradford Method. Dilute sample 1:20 in MilliQ water. Make 75 mg aliquots. Store aliquots at -80C.

ECGS Quantification by Bradford Method
5/20/16

1.
Dilute 5X Protein Assay Buffer (Bio-Rad, stored at 4C) to 1X
Make enough for X number of samples
e.g. standard 5 samples (BSA high 1:10, med 1:20, low 1:100, ECGS #1, ECGS #2) means
6 mL PAB/ 24 mL ddH2O (make extra for pipetting errors)
5 mL per sample

2.
Prepare eppendorf tubes
Add 100 uL each sample diluted in H2O, **add ECGS in hood** (otherwise not sterile)
e.g. for 1:20 dilution add 95 uL H2O and 5 uL sample

3.
Add each 100 uL prepped sample to 5mL PAB tubes
Vortex immediately
Sit 5 min-1hr

4. Read on plate reader, select absorbance and wells
200 uL sample per well
1X PAB, Sample 1, Sample 2, Sample 3, … Sample n