**MEM-based Neuron Growth Medium (new 2/20/17)**

**Overview:**

Earle’s MEM

2.38 mM Sodium Bicarbonate (100x stock in -20)

2 mM Glutamine (Glutamax version from GeminiBio; 100x stock in -20)

0.4% Glucose (100x stock in -20)

0.1 mg/ml Apo-transferrin (Gemini; brown tube, 4C)

5% FBS (-20)

1:50 Gem21 (GeminiBio; -80)

**Protocol for 1L:**

1. Thaw out 100x aliquots of Glucose, Glutamine and sodium bicarbonate (-20, labeled 15 ml conicals). Thaw out 1 50 mL tube of FBS (-20). Thaw out 2 bottles of Gem21 (-80).

2. Remove a total of 100 mL of MEM and put 25 mL into 4 50 mL conical tubes (these tubes will be used to dissolve Matrigel).

3. 1 tube of Apo-transferrin contains 100 mg, so you need to add 1 tube of transferrin to 1 L of media. To dissolve the transferrin, transfer 10 ml of MEM to a 15 ml conical, add the entire contents of the 100mg transferrin tube (make sure to rinse tube). Pipet to make sure transferrin dissolves completely.

4. You will need 2 bottles of MEM to make 1L. To one of the bottles (the one you removed 100ml from) add: 50ml FBS, 10 ml each of glucose, glutamine, sodium bicarbonate. Add both bottles of Gem21. Add 10ml of transferrin solution (step 3).

5. Filter sterilize into 1L steri-top.

6. Cover with foil to prevent the degradation of light-sensitive Gem21.

7. Store at 4C.