**Shelton’s Growth Media (SGM)**

(Revised by Ahna Skop; see Shelton and Bowerman, 1996)

Start by making Inulin and PVP, if you haven’t in the last month. Check also that the Schneider’s and Lipid Concentrate are contamination free and are less than 3 months old.

**Inulin:**

 5 mg/ml in tissue culture dH2O

* 0.2 g / 40 ml (Inulin is stored on the chemical shelf at RT.)
* Autoclave to get into solution. Keeps for 1 month at 4 °C.
* Make sure you write the date made on the bottle.

**PVP:**

 50 mg/ml in Schneider’s Medium

* 0.2 g / 4 ml of Schneider's Medium (PVP is stored on the chemical shelf at RT.)
* Open Schneider’s Medium stock in TC hood only. Get out the 8 ml you need below at the same time that you dispense out the 4 ml you need to dissolve the PVP. NOTE: Look at the Schneider’s before using. The glutamine in the Schneider’s may need to be re-dissolved at 37 °C prior to use. (Leave at 37 °C for 5-10 minutes only.)
* You can stir it with a stir bar you have rinsed with ethanol and dried.
* Keeps in 4 °C for 1 month.
* Make sure you write the date made on the tube.

**Shelton’s Growth Medium:**

8 ml Drosophila Schneider’s Medium (Invitrogen 21720) 4 °C

1 ml 5 mg/ml Inulin (Sigma I3754) **Fresh**

1 ml PVP (MW 40,000) 50 mg/ml (Sigma P0930) **Fresh**

100 μl BME Vitamins (B6891) -20 °C, frost free

50 μl Penicillin-Streptomycin (Invitrogen 15140) -20 °C, frost free

100 μl Lipid Concentrate (Invitrogen 11905) 4 °C

50 μl tissue culture grade dH2O RT

**Filter sterilize.**

**Store at 4 °C in 325 μl aliquots.**

**Good for one week to one month.**

**Keep all solutions in the dark.**

**Before Use:**

Add FBS-ES qualified (heat inactivated @ 56 °C for 30 min) to 35% of volume.

Store in 175 μl aliquots @ -20 °C, frost free. FBS (Gibco 16141-061)

**When ready to use, add mixture and FBS together (325 μl + 175 μl).**

Store at 4 °C. This final solution may be good for one day only, once mixed.

The FBS and the Schneider’s Medium break down rapidly. They also get contaminated VERY easily. Several components are light sensitive, so protect from light. The quality of the serum is critical. It is possible that some batches of FBS will not ever work properly in this recipe.