# SPOC 1 Media "Rat Media" and Cell Culturing

July 2010

Base Medium: GIBCO - DMEM/F12 (w/ glutamine + 15 mM Hepes) #11330-032

| Additives for 500 mL | Stock Concentration | Final Concentration | Product Cat.# |
|---|---|---|---|
| | | | |
| 0.5 mL Insulin | 10mg/ml | 10 μg/ml | Sigma I882 |
| 0.5 mL Hydrocortisone | 0.1 mg/mL, 0.3mM | 0.1 μg/ml | Sigma H0888 |
| 5 mL BPE | | 1% | Pel Freeze |
| 0.5 mL cholera toxin | 0.1 mg/ml | 0.1 μg/ml | Sigma C8052 |
| 0.5 mL Transferrin | 5 mg/ml | 5 μg/ml | Sigma T0665 |
| 0.5 mL Phosphoethanolamine | 50 mM | 50 μM | Sigma P0503 |
| 0.5 mL Ethanolamine | 80 mM | 80 μM | Sigma E0135 |
| 0.5 mL EGF | 25 μg/ml | 25 ng/ml | BD 354001 |
| 3.75 mL HEPES, pH 7.2 | 1M | 30 mM | Sigma H9136 |
| 1.67 mL BSA-LOW Protein | 150 mg/mL | 0.5 mg/mL | Sigma A7638 |
| 0.5 mL Pen/Strep | 1000X | 100 U/0.1 mg/mL | |
| *Retinoic Acid | $5 \times 10^{-5}$M | $5 \times 10^{-8}$M | Sigma R2625 |

*Retinoic Acid: Not absolutely necessary as per Randell et al (AmJRespCellMol Biol, 14:146-154)

**Additive Stock Solutions are stored -20, good for 6 months**

- **Insulin**: 1000x stock = 10 mg/ml in acidified water (0.35 mL glacial acetic acid per 10 ml water)
- **Hydrocortisone**: 1000 x stock = 0.1 mg/ml (make 2 mg/mL stock in abs. EtOH, dilute 1:20 with PBS, =0.3 mM)
- **Cholera Toxin**: 1000 x stock = 0.1 mg/ml in PBS
- **Transferrin** (human): (substitute-BD# 3540204 10 mg): 1000 x stock = 5 mg/mL in PBS
- **Phosphoethanolamine**: 1000 x stock (0.05 M) = 0.071 g/10 mL PBS
- **Ethanolamine**: 1000 x stock (0.08 M) = 48 μL /10 mL PBS
- **HEPES**: stock = 1 M in F-12, pH 7.2 (sterile filter, store at +4)
- **BSA** (Sigma A-7638, essentially globulin free): 150 mg/mL in F-12 or PBS (dissolve gently without making lots of foam, freeze in aliquots)
- **EGF** (mouse): stock 25 μg/mL PBS
- **BPE** (pituitaries are from Pel-Freez Biologicals) preparation- KEEP AS STERILE AS POSSIBLE, let thaw at $4^{o}$ O/N or RT, a few hours, drain blood, rinse with PBS and transfer to another, tared, container weigh and add 2ml of PBS (Ca,Mg free, cold) per gm of tissue, pre-chill to $4^{o}$ mince in blender (Hamilton Beach Blendmaster 7 Speed or equivalent), fill vessel no more than 2/3, start on blend setting holding down lid, run for approximately 30 seconds or until it settles down, then run 10 min on chop setting, in cold room. Aliquot into 500 ml Beckmann tubes and centrifuge @ 5000 rpm in Beckmann JA-10 Rotor 10 min, $+4^{o}$C combine supernatants and freeze in 5mL and/or 30 mL aliquots depending if you need to make 500 ml or 3 L batches.

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For 500 mL medium: thaw 5 mL aliquot, centrifuge @ 3000 g, 10 min., add supernatant to **0.45** μM bottle top filter as last few mLs of media are going through. Some lots are very difficult to filter, in this case pre-filter the BPE supernatant with a 0.8 μM 25 mm syringe filter (Gelman #4618) into the last few mLs of the media as it is going through the bottle top filter.

For 3 L of media, add 3000 g, 10 min. supernatant of 30 mL aliquots directly to media and filter through 0.45 μM capsule filter using peristaltic pump.

We have used commercial BPE for human cells with success! (Sigma: P-1476 at ~14mg/ml- check concentration of each lot; So for 1 L add 0.714 mL of BPE) It is already 0.2 μM filtered, so you can avoid the filtration hassles of homemade BPE. We use it at a concentration of 0.01 mg/mL for human but it has not been tested for SPOC1 or RTE.

***RETINOIC ACID** - MINIMIZE EXPOSURE TO LIGHT

Make stock solution of 1 x $10^{-3}$M in absolute EtOH, store at -80$^{o}$C (for 1000 x stock, dilute this stock 1:20 into F-12-1% BSA(use Sigma BSA- A2058) = 5 x $10^{-5}$M), use 1:1000 (0.5 mL/500 mL) dilution in medium to obtain 5 x $10^{-8}$M.

- check concentration of stock using spectrophotometry
  - molar extinction coefficient = 44,300 at 350 nm in Absolute EtOH
  - Dilute 1 x $10^{-3}$M stock 1: 100 for measurement- abs should equal 0.44, if significantly lower, adjust dilution of 1000x stock accordingly.

# Growing SPOC1 Cells

Thaw the vial of cells quickly by swirling in 37$^{o}$C water bath

Transfer cells to a 15 mL conical and slowly add 5 mL of "Rat Media"+ 2% FBS
**(SPOC1 likes serum when first thawed)**

Spin cells at 500 x G for 5 minutes, aspirate super and gently resuspend pellet in 1-10 mL of rat media + 2% FBS (depending on size of pellet)

Perform cell count& viability (trypan blue)

Plate 1 x $10^{6}$ cells in 100 mm tissue culture dish with 10 mLs of rat media + 2% FBS

After 48 hr, change media to rat media + 2% FBS or serumless rat media (your choice, grows faster in serum)

Change media every 2 days or when media yellows

-split 1:4 after 70% confluent (should be 4-5 days)

Freeze 50% of cells for posterity (or in case of fiasco) in two aliquots in rat media with 10% FBS and 10% DMSO