DIFFERENCES BETWEEN ALI & BEGM

<table>
<thead>
<tr>
<th>Item</th>
<th>ALI</th>
<th>BEGM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media Purpose</td>
<td>Differentiation</td>
<td>Rapid growth on plastic</td>
</tr>
<tr>
<td>Type Culturing</td>
<td>Air/Liquid on porous membrane supports</td>
<td>Submerged on coated dishes</td>
</tr>
<tr>
<td>Base Media</td>
<td>LHC:DMEM-H (50:50)</td>
<td>LHC (100 %)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Amphoteracin</td>
<td>0</td>
<td>0.25 µg/mL</td>
</tr>
<tr>
<td>EGF</td>
<td>0.50 ng/mL</td>
<td>25 ng/mL</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1.0 mM</td>
<td>0.11 mM</td>
</tr>
</tbody>
</table>

REFERENCE
MEDIA PREPARATION (ALI & BEGM)

MATERIALS:
- DMEM-H with glutamine and pyruvate [Cellgro # MT10-013-CV]
- LHC Basal [Invitrogen, #12677-019]
- Numerous Additives (see below)

PROTOCOL
Practice Sterile Technique throughout this procedure!
This protocol is for making 500 mL or 1L batches.
1. Defrost additives aliquots
2. For ALI combine DMEM-H and LHC in a 1:1 ratio.
3. For BEGM, 100% LHC plus additives.
4. Dispense thawed additives into the media. (FOR BEGM, AMPHO is added after filtering) STIR MEDIA
5. Filter media using 0.2 μM filter unit. Store at 4°C.

### ADDITIVES for BEGM and ALI

<table>
<thead>
<tr>
<th>Additive and its Storage Temp.</th>
<th>Stock Conc.</th>
<th>Solution Storage</th>
<th>Final Conc. BEGM</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (INS)</td>
<td>0.87 mM</td>
<td>-20°C</td>
<td>0.87 μM</td>
<td>Sigma I6634</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>0.21 mM</td>
<td>-20°C</td>
<td>0.21 μM</td>
<td>Sigma H0396</td>
</tr>
<tr>
<td>*Epidermal Growth Factor</td>
<td>25 μg/mL</td>
<td>-20°C</td>
<td>25 ng/mL (BEGM)</td>
<td>Invitrogen PHG0313</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.50 ng/mL (ALI)</td>
<td></td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>10 μM</td>
<td>-20°C</td>
<td>0.01 μM</td>
<td>Sigma T6397</td>
</tr>
<tr>
<td>Transferrin</td>
<td>0.125 mM</td>
<td>-20°C</td>
<td>0.125 μM</td>
<td>Sigma T0665</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>2.7 mM</td>
<td>-20°C</td>
<td>2.7 μM</td>
<td>Sigma E4250</td>
</tr>
<tr>
<td>Phosphorylethanolamine</td>
<td>0.5 mM</td>
<td>-20°C</td>
<td>0.5 μM</td>
<td>Sigma P0503</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>0.5 mM</td>
<td>-20°C</td>
<td>0.5 μM</td>
<td>Sigma E0135</td>
</tr>
<tr>
<td>Bovine Pituitary Extract</td>
<td>Check Sigma Lots</td>
<td>-20°C</td>
<td>Sigma 10 μg/mL</td>
<td>Sigma P1476</td>
</tr>
<tr>
<td>Bovine Serum Albumin,</td>
<td>150 mg/mL</td>
<td>-20°C</td>
<td>0.5 mg/mL</td>
<td>Sigma A7638</td>
</tr>
<tr>
<td>Trace Elements</td>
<td></td>
<td>RT</td>
<td></td>
<td>Various products</td>
</tr>
<tr>
<td>Stock 4</td>
<td>1,000 X conc. sol.</td>
<td>-20°C</td>
<td></td>
<td>Various Reagents</td>
</tr>
<tr>
<td>Stock 11</td>
<td>3 mM</td>
<td>RT</td>
<td>3.0 μM</td>
<td>Sigma Z0251</td>
</tr>
<tr>
<td><strong>Penicillin/Strep</strong></td>
<td>1,000 X conc. sol</td>
<td>-20°C</td>
<td>100 U/mL Pen 100 μg/mL Strep</td>
<td>Sigma P3032</td>
</tr>
<tr>
<td>***Amphotericin B,</td>
<td>250 μg/mL</td>
<td>-20°C</td>
<td>0.25 μg/mL</td>
<td>Cellgro MT30003CF</td>
</tr>
<tr>
<td>***Gentamicin,</td>
<td>50 mg/mL</td>
<td>RT</td>
<td>50 μg/mL</td>
<td>Cellgro MT30005CR</td>
</tr>
<tr>
<td>Retinoic Acid</td>
<td>5 X 10⁻⁵ M</td>
<td>-20°C</td>
<td>5 X 10⁻⁸ M</td>
<td>Sigma R2625</td>
</tr>
</tbody>
</table>

*ALI concentration of EGF is 0.50 ng/ml  
**Not in BEGM  
***Not in ALI
**STOCK 11**  
**MATERIALS:**  
- Zinc Sulfate (ZnSO₄·7H₂O) – [Sigma: Z0251](#)  
- Deionized H₂O  
**PROTOCOL:**  
1. Add 0.863 g zinc sulfate to a 1 liter volumetric flask  
2. Add dH₂O to the liter mark  
3. Using a 0.2 μM filter, filter solution in a laminar flow hood and store at RT  

**STOCK 4 (1000X STOCK)**  
**PROTOCOL:**  
1. Add chemicals to a 1 liter volumetric flask  
2. Using a 1 liter volumetric flask, fill to the liter mark with dH₂O + 5 mL HCL.  
3. Filter solution in a laminar flow hood using 0.2 μM filter and store −20°C.

| MATERIALS and AMOUNT PER LITER: |  
|---|---|---|  
| **Component** | **Amount** | **Final Stock Conc.** |  
| Ferrous Sulfate - FeSO₄·7H₂O. | 0.42 g | 1.5 x 10⁻⁶ M |  
| Magnesium Chloride - MgCl₂·6H₂O. | 122.0 g | 6.0 x 10⁻⁷ M |  
| Calcium Chloride - CaCl₂·2H₂O. | 16.17 g | 1.1 x 10⁻⁴ M |  
| [Sigma C3881](#) Hydrochloric Acid | 5.0mL |  

**Ethanolamine**  
**MATERIALS:**  
Ethanolamine (2-Aminoethanol) [Sigma: E-0135](#)  
PBS (1X)  
**PROTOCOL**  
Combine Ethanolamine with PBS in the ratio:  
6.0 μL Ethanolamine/ 200 mL PBS  
Store at −20°C.

**Bovine Pituitary Extract**  
**MATERIALS:**  
[Sigma: P1476](#)  
Check lot concentration from Sigma. Final concentration in BEGM/ALI: 10 μg/mL

**Hydrocortisone (HC)**  
**MATERIALS:**  
Hydrocortisone powder - [Sigma: H0396](#)  
- dH₂O  
- CHECK purity  
**PROTOCOL**  
Dissolve Hydrocortisone powder in dH₂O for a stock concentration of 0.21 mM and store at −20°C.
TRACE ELEMENTS

MATERIALS:
- Individual metal stock solutions (see below)
- Hydrochloric Acid (HCl conc.)
- Deionized water

PROTOCOL:
1. Make separate 100 mL stock solutions of all trace elements as specified in table below
2. Using a volumetric 1 liter flask, fill to the liter mark with H₂O
3. Remove 8 mL of H₂O.
4. Add 1.0 mL of each stock solution listed above.
5. Add 1.0 mL of HCl (12 N).
6. Filter solution using a 0.2 µm filter into a sterile 1L bottle and store at RT.

<table>
<thead>
<tr>
<th>Component</th>
<th>Sigma Cat. #</th>
<th>Amount/100 ml</th>
<th>Molarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium (NaSeO₃) <strong>Highly Toxic</strong></td>
<td>Sigma:S5261</td>
<td>520 mg</td>
<td>30.0 mM</td>
</tr>
<tr>
<td>Manganese (MnCl₂·4 H₂O) <strong>Harmful</strong></td>
<td>Sigma:M5005</td>
<td>20 mg</td>
<td>1.0 mM</td>
</tr>
<tr>
<td>Silicone (Na₂SiO₃·9 H₂O) <strong>Corrosive</strong></td>
<td>Sigma:S5904</td>
<td>14.2 g</td>
<td>500 mM</td>
</tr>
<tr>
<td>Molybdenum [(NH₄)₆Mo₇O₂₄·4 H₂O]</td>
<td>Sigma:M1019</td>
<td>124 mg</td>
<td>1.0 mM</td>
</tr>
<tr>
<td>Vanadium (NH₄VO₃) <strong>Highly Toxic</strong></td>
<td>Sigma: 398128</td>
<td>59 mg</td>
<td>5.0 mM</td>
</tr>
<tr>
<td>Nickel (NiSO₄·6 H₂O) <strong>Toxic</strong></td>
<td>Sigma:N4882</td>
<td>26 mg</td>
<td>1.0 mM</td>
</tr>
<tr>
<td>Tin (SnCl₂·2 H₂O) <strong>Corrosive</strong></td>
<td>Sigma:S9262</td>
<td>11 mg</td>
<td>500 µM</td>
</tr>
</tbody>
</table>

Triiodothyronine

PROTOCOL
1. Dissolve 6.5 mg of T3 powder **Sigma T6397** in 5 mL of 0.2 M NaOH
2. Bring up to 1 liter volume in a large beaker with dH₂O-Stir
3. Aliquot and store tubes at –20°C
   - T3 stock solutions are good for 3 months

Epinephrine

MATERIALS:
- 0.1N HCl
- Epinephrine **Sigma E-4250**
- dH₂O

PROTOCOL
1. Allow epinephrine to warm to room temperature
2. Weigh out 50 mg of Epinephrine and add Epinephrine to 10 ml of 0.1 N HCl
3. In another beaker add 90 mL with dH₂O.
4. Add Epinephrine solution and stir vigorously.
5. Aliquot and store at -20°C
**Insulin**

**MATERIALS:**

- Glacial Acetic Acid
- Insulin powder – Sigma: I6634
dH₂O

**PROTOCOL**

Prepare a 1:20 dilution of Glacial Acetic Acid in dH₂O as follows:

- Aliquot 95 mL dH₂O into a beaker
- Add 5 mL Glacial Acetic Acid to dH₂O
- Add 500 mg of Insulin powder to solution-Stir until dissolved
- Aliquot and store at -20°C

**Transferrin**

**MATERIALS:**

- PBS (1X)
- 1g bottle of human, holo, natural Transferrin -Sigma T0665

**PROTOCOL**

1. Dissolve Transferrin in PBS for a final stock concentration of 10 mg/mL.
2. Aliquot and store at -20°C.

**Epidermal Growth Factor (EGF)**

**MATERIAL:**

- Invitrogen #PHG0313

**PROTOCOL:**

Dissolve EGF in PBS for a final stock concentration of 25 μg/mL.
Store aliquots at -20°C.

**Phosphorylethanolamine**

**MATERIALS:**

- O-Phosphorylethanolamine (Colamine phosphoric acid) C2H8NO4P Sigma: P0503
- PBS (1X)

**PROTOCOL**

Phosphorylethanolamine powder is dissolved in PBS for a final concentration 0.5 mM.
Aliquot and store at -20°C.
**Retinoic Acid**

**MATERIALS:**
- 50 mg of Retinoic acid powder [Sigma- R-2625]
- 100% Absolute Ethanol
- 1 X PBS
- Bovine Serum Albumin [Sigma A2058]

**PROTOCOL:**
- Always keep retinoic acid (RA) solutions on ice and away from light
- **To prepare concentrated 10^{-3} M stock:**
  - Combine 50 mg retinoic acid with 160 mL A. Ethanol for a 1 x 10^{-3} M stock solution.
  - Wrap in foil to protect from light.
  - Store at -80°C
- **To prepare 1000X stock**
  - Confirm concentration of RA stock solution by diluting it 1:100 in Absolute Ethanol.
  - Read the absorbance at 350 nm using spectrophotometer and 1 cm light path quartz cuvet, blanked on 100% ETOH.
  - The molar extinction coefficient of retinoic acid in ethanol equals 44,300 at 350 nm, so the absorbance (O.D.) using a cuvette with a 1 cm path length should equal 0.44 at 350nm.
    - If the absorbance is 0.44, the desired volume of stock solution would be 3.0 ml.
    - If absorbance is not 0.44, the desired volume must be calculated.
      - $DV = \frac{1.35}{Abs}$
      - For example, if O.D. is 0.82, one must add 1.64 ml of RA
    - Combine 4 ml of BSA stock solution with 50 ml of PBS in a 100 ml beaker
    - Add the previously calculated amount of RA stock
    - Bring to a final volume of 60 mL with more PBS
    - Store tubes at -20°C.

**Gentamicin**

**MATERIALS:**
- Gentamicin at 50mg/mL [Cellgro/Fisher # MT30-005-CR]

**Used in BEGM ONLY**

**Amphotericin B**

**MATERIALS:**
- Amphotericin-B at 250μg/mL [CellGro/Fisher # MT-3003CF]

**Used in BEGM ONLY**
**Bovine Serum Albumin (BSA)**

**MATERIALS:**
- Bovine Serum Albumin: Sigma A7638
- PBS (1X)

**PROTOCOL**
1. Directly add PBS to the container of BSA powder to yield a concentration of >150 mg/mL.
2. Gently rock BSA at 4°C for several hours until dissolved.
3. Transfer BSA solution to a graduated cylinder and set volume to yield a final concentration of 150 mg/mL.
4. Filter using a 0.2 µM filter and store at -20°C.

**Penicillin/Streptomycin**

**MATERIALS:**
- Penicillin-G Sodium: Sigma P3032
- Streptomycin Sulfate: Sigma S9137

**PROTOCOL**
1. Dissolve Penicillin G Sodium and Streptomycin Sulfate in dH₂O for a final concentration of 100,000 unit/mL and 100 mg/mL, respectively.
2. Filter and store at .2µM at -20°C.

*Used in ALI ONLY*