

ALI and BEGM

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DIFFERENCES BETWEEN ALI & BEGM

Item	ALI	BEGM
Media Purpose	Differentiation	Rapid growth on plastic
Type Culturing	Air/Liquid on porous membrane supports	Submerged on coated dishes
Base Media	LHC:DMEM-H (50:50)	LHC (100 %)
Gentamicin	0	50 µg/mL
Amphotericin	0	0.25 µg/mL
EGF	0.50 ng/mL	25 ng/mL
CaCl ₂	1.0 mM	0.11 mM

REFERENCE

Lechner JF and LaVeck MA. 1985. A serum-free method for culturing normal human bronchial epithelial cells at clonal density. J. Tissue Culture Methods 9: 43-48.

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 μ M filter unit. Store at 4°C.

ADDITIVES for BEGM and ALI

<u>Additive</u> and its <u>Storage Temp.</u>	<u>Stock Conc.</u>	<u>Solution Storage</u>	<u>Final Conc. BEGM</u>	<u>Source</u>
Insulin (INS)	0.87 mM	-20°C	0.87 μ M	Sigma I6634
Hydrocortisone	0.21 mM	-20°C	0.21 μ M	Sigma H0396
*Epidermal Growth Factor	25 μ g/mL	-20°C	25 ng/mL (BEGM) 0.50 ng/mL (ALI)	Invitrogen PHG0313
Triiodothyronine	10 μ M	-20°C	0.01 μ M	Sigma T6397
Transferrin	0.125mM	-20°C	0.125 μ M	Sigma T0665
Epinephrine	2.7 mM	-20°C	2.7 μ M	Sigma E4250
Phosphorylethanolamine	0.5 mM	-20°C	0.5 μ M	Sigma P0503
Ethanolamine	0.5 mM	-20°C	0.5 μ M	Sigma E0135
Bovine Pituitary Extract	Check Sigma Lots	-20°C	Sigma 10 μ g/mL	Sigma P1476
Bovine Serum Albumin,	150 mg/mL	-20°C	0.5 mg/mL	Sigma A7638
Trace Elements		RT		Various products
Stock 4	1,000 X conc. sol.	-20°C		Various Reagents
Stock 11	3 mM	RT	3.0 μ M	Sigma Z0251
**Penicillin/Strep	1,000 X conc. sol	-20°C	100 U/mL Pen 100 μ g/mL Strep	Sigma P3032 Sigma S9137
***Amphotericin B,	250 μ g/mL	-20°C	0.25 μ g/mL	Cellgro MT30003CF
***Gentamicin,	50 mg/mL	RT	50 μ g/mL	Cellgro MT30005CR
Retinoic Acid	5 X 10 ⁻⁵ M	-20°C	5 X 10 ⁻⁸ M	Sigma R2625

*ALI concentration of EGF is 0.50 ng/ml

**Not in BEGM

***Not in ALI

STOCK 11

MATERIALS:

- Zinc Sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) – **Sigma: Z0251**
- Deionized H_2O

PROTOCOL:

1. Add 0.863 g zinc sulfate to a 1 liter volumetric flask
2. Add dH_2O to the liter mark
3. Using a $0.2 \mu\text{M}$ 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 dH_2O + 5 mL HCL.
3. Filter solution in a laminar flow hood using $0.2 \mu\text{M}$ filter and store -20°C .

MATERIALS and AMOUNT PER LITER:

<u>Component</u>	<u>Amount</u>	<u>Final Stock Conc.</u>
Ferrous Sulfate - $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$.	0.42 g	$1.5 \times 10^{-6}\text{M}$
Magnesium Chloride - $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$.	122.0 g	$6.0 \times 10^{-4}\text{M}$
Calcium Chloride - $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. (Sigma C3881)	16.17 g	$1.1 \times 10^{-4}\text{M}$
Hydrochloric Acid	5.0mL	

Ethanolamine

MATERIALS:

Ethanolamine (2-Aminoethanol) **Sigma: E-0135**
PBS (1X)

PROTOCOL

Combine Ethanolamine with PBS in the ratio:

$6.0 \mu\text{L}$ Ethanolamine/ 200 mL PBS

Store at -20°C .

Bovine Pituitary Extract

MATERIALS:

Sigma: P1476

Check lot concentration from Sigma. Final concentration in BEGM/ALI: $10 \mu\text{g/mL}$

Hydrocortisone (HC)

MATERIALS:

Hydrocortisone powder - **Sigma: H0396**

- dH_2O
- CHECK purity

PROTOCOL

Dissolve Hydrocortisone powder in dH_2O 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.

<u>Component</u>	<u>Sigma Cat. #</u>	<u>Amount/ 100 ml</u>	<u>Molarity</u>
Selenium (NaSeO ₃) Highly Toxic	Sigma:S5261	520 mg	30.0 mM
Manganese (MnCl ₂ · 4 H ₂ O) Harmful	Sigma:M5005	20 mg	1.0 mM
Silicone (Na ₂ SiO ₃ · 9 H ₂ O) Corrosive	Sigma:S5904	14.2 g	500 mM
Molybdenum [(NH ₄) ₆ Mo ₇ O ₂₄ · 4 H ₂ O]	Sigma:M1019	124 mg	1.0 mM
Vanadium (NH ₄ VO ₃) Highly Toxic	Sigma: 398128	59 mg	5.0 mM
Nickel (NiSO ₄ · 6 H ₂ O)- Toxic	Sigma:N4882	26 mg	1.0 mM
Tin (SnCl ₂ · 2 H ₂ O) Corrosive	Sigma:S9262	11 mg	500 µM

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 1×10^{-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.
 $DV = 1.35/Abs$
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 250 $\mu\text{g}/\text{mL}$ **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 μ M 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 .2 μ M at -20°C.

Used in ALI ONLY