HBE1 AIRWAY EPITHELIAL CELL LINE
CULTURE RECOMMENDATIONS

I. **SAMPLES:** Living cells at about 50% confluence in T25 flasks, filled with culture medium. The excess can be decanted, filtered to remove debris, stored at 4^{o}C, and used over the subsequent three weeks.

II. **Medium:** F12+7X medium (see below)

III. **Feeding:** Change medium 2 or 3 times a week, depending on cell density. Use 5 mL for a T25 flask. Doubling time is about 36 hours.

IV. **Passaging:** Passage when 70-90% confluent, or every 7-10 days if not that confluent. Wash cells with Phosphate Buffered Saline (PBS), then treat with 0.1% trypsin + 1 mM EDTA until cells release (usually 5-8 minutes). Neutralize trypsin with 2 fold excess Soybean Trypsin Inhibitor. Maximum expansion is 1:3, and the cells proliferate best if plated at ≥ 30% confluence. We have switched to collagen coated [*Types 1 and 3 collagen, "Purecol", Advanced Biomatrix*] provide better attachment. Proliferation is equivalent on coated and uncoated tissue culture plastic.

V. **Tips:**

Passaging without expansion or with contraction is sometimes useful to stimulate growth.

Expand the lines and prepare cryopreserved stocks before starting experiments. Cryopreserve with 5% FBS and 5%DMSO, freeze at –70^{o}C overnight, then transfer to liquid nitrogen. We freeze 5-6 vials at a time, and thaw one after three days to confirm viability.

To induce polarized differentiation, we seed permeable dishes at confluent density, and supplement medium with 50% (DME +2% FBS that has been conditioned for 3 days by confluent NIH 3T3 fibroblasts).

VI. **REFERENCE:** J. R. Yankaskas, et al., Am. J. Physiol. **264**:c1219-c1230, 1993.

F-12 (7X) Media

Explanation: F-12 (7X) media is prepared by adding 7 hormones to F-12 Media:

Hormone Additive for:	**1 liter of F-12 Media**
Insulin	2.0 mL
Transferrin (TF)	2.0 mL
Hydrocortizone (HC)	1.0 mL
ECGS	1.0 mL
T3	1.0 mL
EGF	1.0 mL
Cholera Toxin	100 μL

Preparation of F-12 Media

MATERIALS:

- 53.15g F-12 Nutrient Mixture (Ham) powder with L-glutamine without Sodium Bicarbonate (Gibco-21700091). Store powder at 4°C
- 50 mL 1.5 M Hepes, stored at 4°C
- 100 mL 0.714 M $NaHCO_3$. Solution stored at 4°C
- 4 mL Gentamicin (Sigma G-1522, 50mg/mL). Solution stored at 4°C.
- 2.5mL Pen/Strep 1000X (Stock concentration 100,000 U/mL and 100 mg/mL respectively). Solution stored at -20°C

PROTOCOL:

1. Add 53.15g F-12 powder to approximately 4 liters H_2O in a 6L flask.
2. Stir on unheated stir plate at medium speed.
3. After F-12 powder has gone into solution, add 50 mL of 1.5 M Hepes and 100mL of 0.714 M $NaHCO_3$ and stir.
4. pH media to 7.2 using 1 M HCl or 1 M NaOH.
5. Add 4 mL Gentamicin and 2.5 mL Pen/Strep 1000X and stir.
6. Bring solution to final volume of 5 L in a graduated cylinder, with stirring, by adding deionized/distilled H_2O.
7. Filter using a 0.2μ*M* filter.
8. Store bottles at 4°C up to 4 weeks.

Note: If F12 Nutrient powder does not contain L-glutamine, add 730 mg/5 l of L-glutamine. Final concentration of L-glutamine will be 1m*M*.

HORMONE PREPARATION AND STORAGE

Hormone/Source	**Stock Prep.**	**Stock Conc.**	**Storage/ Expires?**	**Final Conc. in F12-7X**
Insulin Sigma I-6634	20 mg/4 mL dH2O	5 mg/ml	-20°C/ 3 mos.	10 μg/mL
HC (Hydrocortisone) Sigma H-0888	1) 50 mg/13.8 mL 70% ETOH 2) dilute 1:10 in PBS	10^{-2} M 10^{-3} M	1) -20°C/ 1 yr 2) -20C/ 3 mos.	10^{-6}M
ECGS (Endothelial Cell Growth Supplement) Sigma E2759	15 mg/4 ml dH2O	3.75 mg/ml	-20°C/1 mo.	3.75 μg/ml
EGF (Epidermal Growth Factor)/ Sigma E4127	100 ug/4 mL dH20	25 μg/ml	-20°C/3 mos.	25 ng/ml
T3 (Triiodothyronine) Sigma T-6397	1) 20 mg/5 mL 0.2M NaOH 2) add 5 mL dH2O 3) dilute 1:100 in dH2O	3 X 10^{-5} M	-20°C/3 mos.	3 X 10^{-8}M
Transferrin Sigma T0665	10 mg/4 mL dH2O	2.5 mg/ml	-20°C/3 mos.	5 μg/ml
Cholera Toxin Sigma C-3012	0.5 mg/5 mL dH2O	100 μg/ml	4C°/1 yr	10 ng/ml