

MOUSE TRACHEAL EPITHELIAL CELL (mTEC) ISOLATION

- By Mary Brummet

Day 1

Dissection:

1. Euthanize with Ketamine/Xylazine, 0.15-0.18ml/mouse
2. Rinse whole body with 70% ethanol
3. Place with abdomen up and cut open the peritoneal cavity
4. Work from the head down; cut the hide just over the trachea. Using clean scissors cut open the tissue above the trachea to expose it. Note: watch for the two longitudinal arteries alongside the trachea, this will lead to a lot of blood.
5. Place scissors under the trachea and cut the connective tissue and release the trachea.
6. Cut the trachea above the larynx and then cut down into the lungs, just below the bronchi
7. Place the trachea in a 50ml conical tube containing Ham's F-12 with pen/strep. Keep on ice.

Cell Recovery:

All subsequent steps are performed in a sterile tissue culture hood

8. Fill 3-P100 tissue culture dishes with cold Ham's F-12 pen/strep, and place tracheas in first dish
9. Using fine tipped forceps, clean off the connective tissue around the trachea. After cleaning, place into the next P100 dish
10. Repeat cleaning steps until the trachea is cleaned (as much connective tissue removed as possible)
11. Using small scissors, cut most of the larynx off (the yellowish part), and cut the tracheas open lengthwise.
12. Place the tracheas in a 15ml tube containing 0.15% pronase, diluted in the F-12 P/S media. Add enough to over the tracheas and incubate 4°C overnight.

Plate preparation:

13. Collagen-coat transwell inserts (usually one insert per trachea) and allow to dry overnight, room temperature, in the sterile hood with the UV light on.

Day 2

Cell Recovery and Plating:

14. The next morning gently invert the tube containing the tracheas 3-5 times and set up to harvest cells
15. Add FBS to a final concentration of 10% to the tube and invert 20-30 times
16. Place the tracheas in a 15ml conical containing 5ml of Ham's F12 p/s +10% FBS. Invert tube 20-30 times
17. Repeat step 16.
18. Remove tracheas and combine all washes into one 15ml conical.
19. Pellet cells, 1200rpm, 5 min, 4°C.
20. Carefully aspirate the supernatant and re-suspend the cells in DNase solution (200ul/mouse trachea). Do this very gently so as to not damage the cells. Gently flick to loosen the pellet. Pipet up and down 4-5 times.
21. Place DNase digestion at room temperature for 10 min and then pellet the cells, 1200rpm, 10min, 4°C
22. Resuspend pellet in TEC Basic media + 10% FBS and place in a tissue culture dish (1 trachea/6mm dish), incubate 37°C, 5% CO₂, 3-4 hours to allow fibroblasts to attach.
23. While incubating, wash the collagen coated wells 3X with PBS.
24. After incubation, carefully collect the un-adhered cells in the supernatant and pellet in a 15ml conical, 1200rpm, 5 min, 4°C
25. Carefully remove the supernatant and re-suspend the pellet in pre-warmed TEC Plus/RA media. (use 100ul/trachea). Be gentle with pipetting to break up the clumps.
26. Count the cells using a hemacytometer and trypan blue exclusion. Viability should be >90%. Yield may vary but typically 1x10⁵ cells/mouse trachea is good.
27. Plate cells at a density of of 1x10⁵ cells/cm².
28. Place 500ul of TEC Plus/RA in basolateral chamber
29. Place at 37°C, 5%CO₂, this is day 0 of culture.
30. Change media day 3
31. Check resistance every 3 days
32. **Note 1:** There will be a little liquid in the apical chamber, this is mostly mucus.
33. **Note 2:** Ciliated cells will be visible at day 5 ALI and become very prominent by day 10

TEC MEDIA FORMULATIONS

Media	Components	Amount
TEC Basic	1M Hepes	7.5ml
	200mM Glutamine	10ml
	7.5% NaHCO ₃	2ml
	Fungizone (1000X, 250ug/ml)	500ul
	P/S (100X, 10,000U or ug/ml)	5ml
	Add DMEM/F-12 50:50 mix to a volume of 500ml	
	Sterile filter, store 4°C, good for 4 weeks	
TEC Plus	Insulin (10ug/ml final)	1250ul
	Transferrin (5ug/ml final)	250ul
	Cholera Toxin (0.1ug/ml final)	250ul
	Epidermal growth factor(25ng/ml final)	1250ul
	Bovine Pituitary extract (15mg/500ml)	2.5ml
	FBS (5% final)	12.5ml
	Add TEC Basic to 250ml Add RA (100x stock) fresh Sterile filter, store 4°C, good for 2 weeks	
TEC MM	Add Nuserum to TEC basic to 2% final concentration Add RA (100X) prior to media change	
RA (1x10 ⁵ stock)	Powder	50mg
	95% ethanol	33.3ml
	Light Sensitive!! Store in the dark, -70°C	
RA (100x stock)	1x10 ⁵ stock solution	50ul
	30% BSA stock (0.1% final)	167ul
	HBSS	50ml
Note: vortex RA stock vigorously before making working stock. Flakes need to go into solution. Light Sensitive!! Store in 1ml aliquots at -70°C		
Rat Tail Collagen	Stock 4.10mg/ml (82x) Dissolve 50ug/ml in 0.02N Acetic Acid Sterile filter, store 4°C	
0.02N Acetic Acid	575 ul of glacial acetic acid into 500ml of water = 0.02N Add 6ml of 4mg/ml Rat collagen Stock to 500ml 0.02N Acetic Acid	
Pronase	0.15% (1.5mg/ml final) Dissolve 15mg of Pronase into 10ml Ham's F-12 p/s	

Sterile filter, make fresh each time

DNase solution	0.5mg/ml final
Stock Solution	100mg in 10ml of Ham's F-12 p/s Sterile filter, store -20°C
Working solution	(0.5mg/ml) 1ml of stock solution into 19ml of Ham's F-12 p/s Sterile filter, store -20°C
Ketamine /Xylazine	Ketamine 100mg/5ml saline + Xylazine 20mg/13ml saline Bring to a final volume of 50ml with saline

Reagent Ordering Information

Rat Tail Collagen Type 1	Becton Dickenson Cat# BD 354236
Retinoic Acid	Sigma Cat# R2625
Transwell Inserts	Fisher Scientific Cat#35-3090
Plates for Transwells	Fisher Scientific Cat# 35-3502
NuSerum	BD Cat# 355100 Need to heat inactivate, 65oC, 15min, then aliquot
Pronase	Roche Applied Sciences Cat# 10165921001 (1g)
DNase	Sigma Cat# DN-25 100mg
DMEM/F-12 Media	Gibco Cat# 11330-032
Ham's F-12	Cellgro Cat# 10-080-cv
HBSS	Gibco Cat# 14175
Ketamine	Sigma Cat# K2753
Xylazine	Sigma Cat# X1251

TEC media-Hormone Reconstitution

<u>Hormone</u>	<u>Stock Conc</u>	<u>Final Conc</u>	<u>Aliquot</u>	<u>AMT/250ml media</u>
<u>Insulin</u>	2mg/ml	10ug/ml	750ul	1250ul
<i>Cat# Sigma I1882: Dissolve 100mg in 50ml 4mM HCL</i>				
<u>Transferrin</u>	5mg/ml	5ug/ml	250ul	250ul
<i>Cat# Sigma T8158: Dissolve 100mg in 20ml HBSS +0.1% BSA (70ul of 30% stock)</i>				
<u>Epithermal Growth Factor</u>	5ug/ml	25ng/ml	1250ul	1250ul
<i>Cat# Sigma E4127: Dissolve 100ug in 20ml HBSS + 0.1%BSA (70ul of 30% stock)</i>				
<u>Cholera Toxin</u>	100ug/ml	0.1ug/ml	250ul	250ul
<i>Cat# Sigma C8052: Dissolve 1mg in 10ml HBSS + 0.1% BSA (33ul of 30% stock)</i>				
<u>Bovine Pituitary Extract</u>	3mg/ml	30ug/ml	1250ul	2500ul
<i>Cat# BD 354123: Dissolve 15mg in 5ml of HBSS, Will be a little cloudy.</i>				