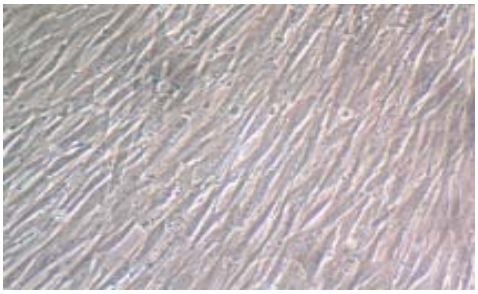
MLPC™-UCB Clonal Cell Line
Respiratory Epithelial Differentiation of MLPC

Reagents and Materials

- MSCGM, Lonza Walkersville, Inc., PT-3001
- Small Airway Growth Media, Lonza Walkersville, Inc., CC-3118
- Lab Tek II 4-well chamber slides, Nalge Nunc, 154526
- Hank's Balanced Salt Solution, Sigma, H1641 or equivalent
- Bovine Serum Albumin, Fisher, BP1600-1 or equivalent
- Paraformaldehyde, Sigma, P-6148 or equivalent
- anti-Prosurfactant Protein C, Chemicon, AB3428
- Alexa Fluor 594 goat anti-rabbit IgG, Moleular Probes, A11072



Control MLPC observed under phase contrast microscopy.

Epithelial Procedure

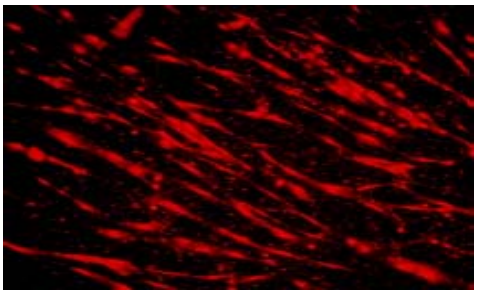
1. Plate MLPCs in a non-coated 4-well chamber slides at 2.0×10^4 cells/well in MSCGM.
2. Incubate the cells in a 5% CO_2 atmosphere at 37°C.
3. Cells are fed every 3-4 days with MSCGM until the cultures reach confluency.
4. At confluency, continue to feed the control wells with MSCGM.
5. The cells to be differentiated have their medium replaced with small airway growth media (SAGM)
6. Continue to feed with the above medias every 3-4 days for approximately 10 days.

MLPC differentiated with Small Airway Growth Media observed under phase contrast microscopy.

Epithelial Staining

1. Remove the medium from each well of the chamber slide.
2. Wash each well twice with 1 mL Hank's Balanced Salt Solution-HBSS supplemented with 1% BSA .
3. Add 1mL of 4% Paraformaldehyde to each well.
4. Incubate for 20 minutes at room temperature.
5. After incubation, remove the 4% Paraformaldehyde and wash the wells twice with 1 mL of HBSS + 1% BSA.
6. Add approximately 500µl of HBSS + 1% BSA to each well.
7. Add the appropriate amount of pro-SPC primary antibody to each well and incubate overnight at 2-8°C.
8. After the overnight incubation, wash each well twice with 1mL of HBSS + 1% BSA.
9. Add approximately 250µl of HBSS + 1% BSA to each well.
10. Add the secondary antibody, Alexa Fluor 594 GAR to each well.
11. Incubate 20 minutes at room temperature.
12. Perform two final 1mL washes with HBSS + 1% BSA to each well. Remove the chamber slide. Cover the slide with a coverslip, retaining some fluid underneath to keep cells moist while examining under fluorescent microscope.

Control MLPC cultures stained for pro-SPC, observed under fluorescent microscopy.



MLPC differentiated with Small Airway Growth Media, stained with pro-SPC and observed under fluorescent microscopy.