3D Airway Differentiation

CnT-Prime Airway medium (CnT-PR-A) is designed to provide strong proliferation and extended longevity of large airway cells in a fully defined environment.

It is a Progenitor Cell Targeted (PCT) medium, which contains additional factors to improve retention of large airway epithelial cells in a proliferative progenitor phenotype, thereby improve proliferation and longevity.

PCT factors may retard the differentiation process. For this reason, it is recommended to switch to CnT-PR-AD + 1 mM $CaCl_2$ differentiation medium when cells are induced to differentiate.

Primary cells at passage 1 generally provide the most complete differentiation, and yield the most extensive cilia development. Cilia are generally visible after 10-14 days, and normally continue to develop for at least 21 days.

We recommend the use of 12mm polycarbonate inserts with a pore size of 0.4 um. We commonly use the Millicell PCF inserts available from Millipore.

To enable more convenient medium changes and handling, a number of inserts may instead be placed in a single 60 mm petri dish during establishment of the models. This is the method described below.

1. Place six inserts into a 60 mm cell culture dish.
2. Wet the inserts with 3 mL CnT-PR-A medium.
3. Directly add 2 x 10^5 cells in 400 µL CnT-PR-A medium per insert (cell suspension stock solution: 5 x 10^5 cells / mL).
4. Add the appropriate amount of CnT-PR-A medium outside the inserts (into the cell culture dish, e.g. ~ 11 mL for cell culture dishes with 53 mm inner diameter, if dishes with another inner diameter are used adjust the quantity of medium accordingly), so that medium levels inside and outside the insert are equal and the cells are submerged.

Important note: make sure that no air bubbles are trapped underneath the membrane for the entire duration of the 3D culture.

5. Place the inserts in a humidified incubator at 37°C and 5% CO_2
6. Grow the cells until they reach confluencey. This is typically 2 days, when using early passage keratinocytes that have been proliferating well in the preceding week.
7. Optional: if you are unsure of the growth rate of the cells, it is advised to stain one insert to examine confluency, as cultures must be confluent before proceeding further with air-lift culture. It is recommended to fix the cells in the insert first (e.g. with 100% MeOH), and then stain with diff-quick, giemsa, or equivalent to allow visualisation of cell confluence.
8. If the monolayer is confluent, proceed with step 9, otherwise change medium in the remaining inserts, cultivate for another day to reach confluence. If confluence is not achieved in 3 days, the growth is unlikely to be sufficient to successfully develop a successful 3D culture.
9. Replace the CnT-PR-A medium with CnT-PR-AD + 1 mM $CaCl_2$ medium inside and outside the insert (same amounts as when seeding, Step 3 & 4).
10. Place the inserts in the incubator overnight (15 to 16 h) to allow cells to start the differentiation process.

11. Initiate 3D cultures by aspirating all the medium from inside the insert and replacing outside medium with fresh CnT-PR-AD + 1 mM $CaCl_2$ differentiation medium up to the level of the membrane (e.g. ~ 3.2 mL for cell culture dishes with 53 mm inner diameter, if dishes with other inner diameter are used, adjust the quantity accordingly to obtain the correct medium level). Three inserts should be used per 60 mm dish (approx 1 mL of medium per insert) to allow 3 medium changes per week (i.e. Mon/Wed/Fri). Using this approach (1 mL medium per insert/medium change 3x per week) models are typically fully established on day 12. If inserts need to be incubated separately (for example in 24 well plates), the volume of medium per insert will be lower, and the medium should be changed daily.
12. Important note: The surface within the inserts should dry following air-lift and should remain dry for the rest of cultivation. Therefore it is very important to have a confluent monolayer before starting the air-lifted culture.
13. For histochemistry, immunohistochemistry or immunofluorescence analyses, 3D-cultures are fixed over night at 4°C by placing the insert in a separate 24-well plate and filling the well and the inside of the insert with 4% Paraformaldehyde / PBS. Cut the skin / membrane out and proceed for paraffin embedding and H & E staining (for detailed information see separate protocol on our resources section).

For more information, please email out scientists directly: scientist@cellntec.com