

## 3D Culture of Lung Alveolar Cells

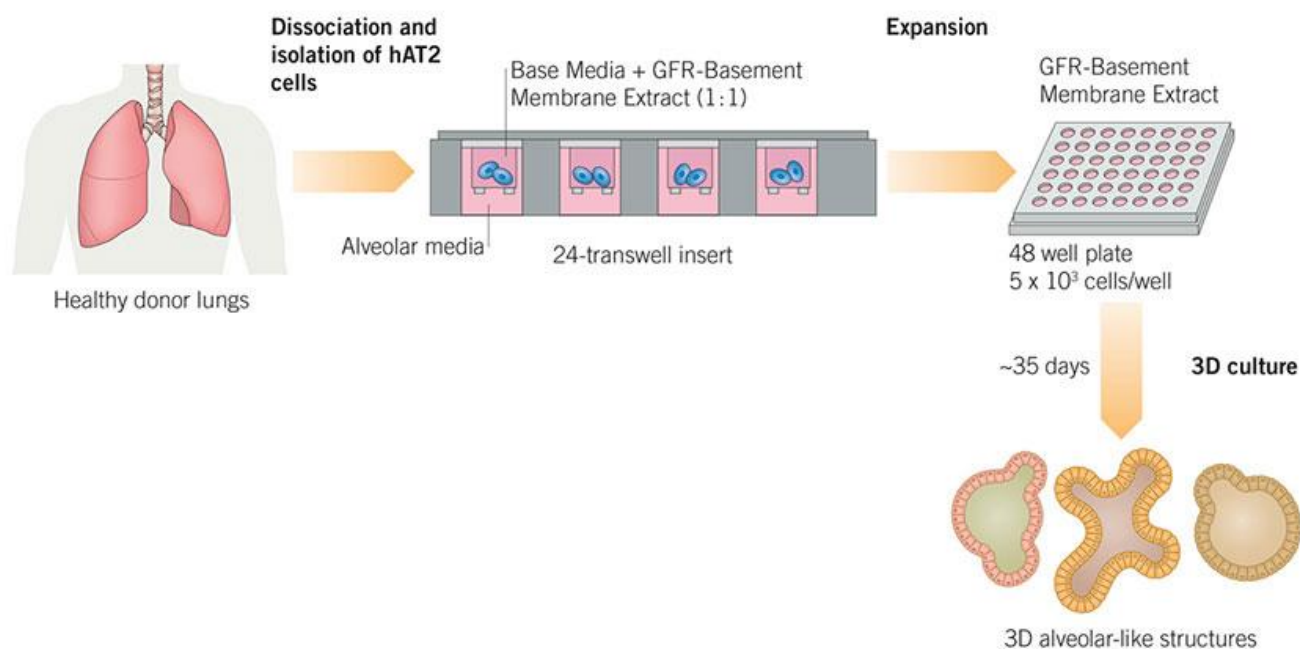
*This is intended as a guide only; for full experimental details please read the reference provided.*

### In Brief

Youk *et al.* describe a protocol for the establishment of feeder-free, long-term 3D cultures of human lung alveolar type 2 (hAT2) cells in chemically-defined conditions and their use in the study of [SARS-CoV-2](#) infection.

Distal parenchymal tissue from healthy donor lungs was dissociated and hAT2 cells were isolated. Isolated HTII-280+ cells were resuspended in a mixture of base media and growth factor-reduced (GFR) basement membrane extract (BME, e.g. [Cultrex™](#)) at a ratio of 1:1 in 24-well transwell inserts. Alveolar media was added to the lower chamber and cells were maintained under standard culture conditions with media changes every 2-3 day. ROCK inhibitor [Y-27632](#) was included in the media for the first 48 h to promote cell survival. For 3D culture, cells were transferred to 48-well plates, and embedded in GFR-BME.

Under these conditions, hAT2 cells self-organize into 3D cellular structures with heterogeneous size and morphology by around day 35, including both folded and cystic-like structures consisting of mature hAT2 cells expressing pro-surfactant protein C (pro-SFTPC) HTII-280, and ABCA3. Cells in 3D cultures could be passaged multiple times and at different days depending on size.



## Cocktails

Base Media		Alveolar Media (Base Media + B27)	
<a href="#">DMEM/F-12</a> Cat.No. M23350		<a href="#">human R-Spondin 1</a> Cat.No. 4645R	10%
<a href="#">HEPES</a> Cat.No. 3173	10 mM	<a href="#">human EGF</a> Cat.No. 236-EG	50 ng/mL
<a href="#">Nicotinamide</a> Cat.No. 4106	10 mM	<a href="#">human KGF</a> Cat.No. 251-KG/CF	100 ng/mL
<a href="#">N-Acetylcysteine</a> Cat.No. 5619	1 mM	<a href="#">human FGF-10</a> Cat.No. 345-FG/CF	100 ng/mL
<a href="#">Penicillin/Streptomycin</a> Cat.No. B21210	1 U/mL	<a href="#">human Noggin</a> Cat.No. 6057-NG/CF	100 ng/mL
		<a href="#">SB 431542</a> Cat.No. 1614	10 µM
		<a href="#">CHIR 99021</a> Cat.No. 442	3 µM
		<a href="#">Y-27632</a> Cat.No. 1254	10 µM (day 1 and 2 only)
		<a href="#">Amphotericin B</a> Cat.No. 6930	250 ng/mL (days 1 -5)
		<a href="#">Gentamicin</a> Cat.No. 6442	50 µg/mL (days 1 -5)

## Reference

Youk *et al.* (2020) Three-dimensional human alveolar stem cell culture models reveal infection response to SARS CoV- 2. *Cell Stem Cell* **27** 1. PMID: [33142113](#)