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Primary Lung Fibroblasts (PLF) Plating/Freezing Protocol

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SUBMIT TO PLOS ONE

ABSTRACT

PLF Media

90 mL DMEM NUTRIENT MIX F12 (Invitrogen/Cell Center, cat# 11320033)

10 mL 10% FBS (Invitrogen, cat#10437-028)

1 mL ANTIBIOTIC ANTIMYCOTIC-Anti-Anti 100x (Invitrogen/Cell Center, cat#15240096)

Freeze Media

40 mL PLF Media

5 mL 10% FBS (Invitrogen, cat# 10437-028)

5 mL DMSO (Sigma, cat#D2650-100ml)

Nunc™ EasYFlask™ Cell Culture Flasks, T75, filter (hermo/ Life Tech, cat#156499)

TRYPsin 0.05% EDTA (Invitrogen/Cell Center, cat#25300054)

CryoVials (Neta Sci, cat#430659)

PROTOCOL CITATION

Morrisey Lab 2021. Primary Lung Fibroblasts (PLF) Plating/Freezing Protocol. [protocols.io](https://protocols.io/view/primary-lung-fibroblasts-plf-plating-freezing-prot-bsyrnfv6)
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- 1 In TC hood label 1-2 T75 flasks with lung ID, date, disease or NI (normal), initials, and number of cells.
- 2 Obtain single cell suspension from lung, count cells, and keep on ice.

- 3 Spin down 10-12 million cells in 4C centrifuge for 5 min at 300xg(rcf).
- 4 Using a 10mL serological pipette (slow settings), resuspend cells with 10 mL of cold PLF media in T-75 flaks/s and put in incubator.
- 5 a. It is important to mix the cells evenly
- 6 After ~24 hours, change PLF media on the cells.
- 7 Change PLF media 2-3 times a week until cells are relatively confluent. They grow very patchy, so it is sometimes hard to tell, but this usually takes about 3 weeks.
- 8 Once confluent, split the cells out into more flasks. Usually 1:6 is OK for the first split, but depending on confluency, this might change.
- 9 a. If you started with 2 plates of cells, can split them each out 1:12
- 10 Once those flaks are confluent (usually 3-4 days later), split 1:4 so you end up with 24 flasks total.
- 11 Once the 24 flasks are confluent (usually 3-4 days later) freeze down cells by trypsinizing the flasks with 0.05% trypsin and resuspending cells in 1 mL of Freeze Media/vial.
- 12 a. It is easiest to label cryo vials, with lung ID, disease/NI, date, initials, before trypsinizing flasks.
- 13 b. There will be a total of 50 vials (resuspend pellet in 50 mL of Freeze media).
- 14 c. There is approx. 1 million cells per vial. Can count cells if you want ,but is not necessary for freezing.
- 15 10. Put vials in a Mr. Freezy and put in -80 for at least 24 hours.
- 16 11. Transfer vials to liquid nitrogen tank and update google sheetàHuman Samples-à liquid nitrogen tank

