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Primary Lung Fibroblasts (PLF) Plating/Freezing Protocol

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1 Works for me This protocol is published without a DOI.

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SUBMIT TO PLOS ONE

ABSTRACT

Primary Lung Fibroblasts (PLF) Plating/Freezing Protocol

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

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Plating and FreezingPLF

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.

4.1 a. It is important to mix the cells evenly

5 After ~24 hours, change PLF media on the cells.

6 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.

7 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.

7.1 a. If you started with 2 plates of cells, can split them each out 1:12

8 Once those flaks are confluent (usually 3-4 days later), split 1:4 so you end up with 24 flasks total.

9 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.

9.1 a. It is easiest to label cryo vials, with lung ID, disease/NI, date, initials, before trypsinizing flasks.

b. There will be a total of 50 vials (resuspend pellet in 50 mL of Freeze media).

9.2

9.3 c. There is approx. 1 million cells per vial. Can count cells if you want ,but is not necessary for freezing.

10 10. Put vials in a Mr. Freezy and put in -80 for at least 24 hours.

11 11. Transfer vials to liquid nitrogen tank and update google sheetàHuman Samples-à liquid nitrogen tank