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Thawing, Expanding, and Freezing Human Fibroblasts

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1 Works for me dx.doi.org/10.17504/protocols.io.bgunjwve

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Thawing Human Fibroblasts

1 Prior to thawing cells, prepare culture media

1.1 hFibroblast Media:

DMEM/F12, 10-20%
FBS
1% L-Glut (or Glutamax)
1% Antibiotic

2 Remove cells from liquid nitrogen storage and thaw quickly by placing vial in a **37 °C** water bath for approximately **00:01:00**

Be sure to monitor cells during thaw and make sure they are removed from water bath just prior to completely thawing

3 Quickly transfer thawed cells into conical tube containing **9 ml** of room temperature DMEM/F12

4 **750 rpm, Room temperature 00:03:00**

5 Aspirate supernatant, be careful not to distrub cell pellet

6 Re-suspend cells in **5 ml** of hFibroblast Media by pipetting cells up and down 2-3 times to achieve a single cell suspension

7 Transfer cell suspension to T25 cell culture flask

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8 Incubate at **37 °C** and 5% C02 overnight

9 Replace hFibroblast Media every 2-3 days

Expanding Human Fibroblasts

10 Upon reaching 80-90% confluency, fibroblasts will create a swirling pattern. This is the appropriate time to split the cells

Confluent fibroblasts can be split from one T25 cell culture flask into one T75 cell culture flask OR two T25 cell culture flasks.

11 Aspirate hFibroblast Media from T25 cell culture flask

12 Wash cells with **5 ml** 1X PBS

13 Add **1 ml** of 0.25% Trypsin to cells. Incubate at **37 °C** for **00:05:00** to allow cells to dissociate from cell culture flask

After incubation period, check cells under a microscope to determine the extent to which cells have detached from the flask. If many cells remain attached, incubate at **37 °C** for an additional **00:05:00** .

14 Re-suspend cells in **5 ml** of hFibroblast Media. Pipet cells up and wash flask 4-6 times to collect all cells and to achieve a single cell suspension.

15 Add cell suspension to **7 ml** of fresh hFibroblast Media and transfer to T75 cell culture flask

16 Incubate at **37 °C** at 5% C02 overnight

17 Change media every 2-3 days until fibroblasts become confluent

At this point fibroblasts can be passaged again, used for experiments, or frozen down and banked

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Freezing Human Fibroblasts

18 Upon reaching 80-90% confluency, fibroblasts will create a swirling pattern. This is the appropriate time to split the cells

It is important to keep fibroblast cultures at a low passage number, thus when thawing cells it should be a priority to expand cells for freezing over expanding cells for experiments.

One T75 cell culture flask will produce enough cells to freeze 2-3 **1 ml** aliquots.

19 Aspirate hFibroblast Media from T25 cell culture flask

20 Wash cells with **5 ml** 1X PBS

21 Add **2 ml** of 0.25% Trypsin to cells. Incubate at **37 °C** for **00:05:00** to allow cells to dissociate from cell culture flask

22 Re-suspend cells in **7 ml** of hFibroblast Media. Pipet cells up and wash flask 4-6 times to collect all cells and to achieve a single cell suspension.

23 Transfer cell suspension to conical tube for centrifugation

24 **500 rpm, Room temperature 00:05:00**

25 Aspirate supernatant, be careful not to distrub cell pellet

26 Re-suspend cell pellet in **1.5 ml** hFibroblast Media.

27 Add **1.5 ml** 2X Freezing Media (FBS, 10% DMSO) to cell suspenion from Step 26.

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This should result in a final volume of **3 ml** which can be equally distributed into **1 ml** aliquots across three cryovials.

28 Transfer **1 ml** to each pre-labeled cryovial

29 Immediately place cells in **-80 °C** freezer for **72:00:00**

30 Transfer cells to long-term liquid nitrogen storage

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