NOV 08, 2023



**DOI:**
dx.doi.org/10.17504/protocols.io.36wgq3edklk5/v1

**Protocol Citation:** Ching-Chieh Chou, Judith Frydman 2023. Human primary fibroblast culture. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgq3edklk5/v1



**Protocol status:** Working
We use this protocol and it's working

**Created:** Nov 08, 2023

# Human primary fibroblast culture

Ching-Chieh Chou[1], Judith Frydman[1]

[1]Department of Biology, Stanford University

ASAP Collaborative Research Network | Ian Chou

Ching-Chieh Chou
Stanford University

ABSTRACT

This is a protocol for human primary fibroblast culture.

**Last Modified:** Nov 08, 2023

**PROTOCOL integer ID:** 90642

**Keywords:** ASAPCRN, Fibroblast

**Funders Acknowledgement:**
Aligning Science Across Parkinson's
Grant ID: ASAP-000282

## Preparing culture medium

1 Fibroblast medium @4°C lasts for 1 month. A total of 500 mL:
- 435 ml DMEM (High glucose, GlutaMAX) (Thermo Fisher Scientific)
- 50 ml Fetal bovine serum (FBS) (Thermo Fisher Scientific)
- 5 ml Penicillin-Streptomycin (Thermo Fisher Scientific)
- 5 ml MEM NEAA (100X; Thermo Fisher Scientific)
- 5 ml Sodium pyruvate (Thermo Fisher Scientific)
- 0.5 ml beta-mercaptoethanol (Thermo Fisher Scientific)
- Sterilized by filtration through a 0.22 µm filter

## Thawing, subculturing and freezing fibroblasts

2 Remove a cryogenic vial containing the frozen human primary fibroblasts from liquid nitrogen storage and thaw in a 37°C bead bath for a few min. Constantly check the content.

3 After the content is thawed, immediately transfer and slowly add to a 15 mL conical tube containing 5 mL of cell culture medium.

4 Centrifuge at 200xG for 5 min to pellet the cells.

5 Remove the supernatant, add 10 mL of fresh culture medium for culturing cells in a 10-cm dish.

Note

If the cell numbers are low, recommend to first culture the cells in a well of a 6-well plate filled with 2 mL of fresh culture medium to improve cell survival and growth.

6 Replace medium every 3-4 days. Fibroblasts should be confluent after one week.

7 For subculturing, rinse the dish with sterile 1x PBS to remove all complete medium before splitting.

8 Add 0.05% Trypsin-EDTA to cover the bottom of the dish. Incubate at 37°C with 5% $CO_2$ for ~5 minutes. Cells should round up and become dislodged.

9 Neutralize trypsin-EDTA activity by adding the complete media.

10 Gently pipette to resuspend the cells and transfer the cell-containing medium to a 15 mL conical tube.

11 Centrifuge at 200xG for 5 min to pellet the cells.

12 Remove the supernatant, add fresh culture medium and split the cells at 1:3 ratio.

13 Replace medium every 3 to 4 days. Fibroblasts should be 80 to 90% confluent after one week and ready for experiments.

> **Note**
>
> Avoid subculture at low-density as it will age the cells.

14 If want to freeze the cells, after centrifugation (Step 11), remove the supernatant, and add 1 to 2 mL of Recovery Cell Culture Freezing Medium (Thermo Fisher Scientific).

> **Note**
>
> Recommend freezing cells at a high concentration.

15 Gently pipette to resuspend the cells and transfer the cell-containing medium to a cryogenic vial. Then store the cryogenic vial in a cryo-freezing container that limits the decrease in temperature at ~1°C per min at -80°C.

16 For long-term preservation, transfer the cryogenic vial from a -80°C freezer to a liquid nitrogen tank.