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Human primary fibroblast culture

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We use this protocol and it's working

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Abstract

This is a protocol for human primary fibroblast culture.

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% CO₂ 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.