PRIMACYT
Cell Culture Technology

Thawing and culture of human fibroblasts

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Version 01

Recommended medium

- FGM: for thawing and culture of human fibroblasts (adults and juvenile)

1. After arrival of the cryo-preserved/plated cells in your laboratory

- Place the cryovial with frozen fibroblasts immediately into the gas phase of liquid nitrogen tank or store at -150°C

- Place the flask with cultured fibroblasts into the 37 °C incubator
- Warm the cell culture medium FGM to 37 °C
- Change the medium in the flask and continue culture until approx. 80 % confluency

2. Thawing of primary human fibroblasts

- Use the FGM as thawing medium
- Warm waterbath and FGM to 37 °C
- Add 5 ml FGM into 15 ml tube
- Remove vial with fibroblasts from liquid nitrogen/-150 °C and place it immediately into the 37 °C warm waterbath until cell suspension is thawed (approx. 2 min)
- Spray 70 % ethanol on the cryovial
- Transfer cell suspension into the tube with 5 ml FGM
- Wash cryotube with 0.5-1 ml FGM to remove the cells completely from the cryovial
- Pellet the fibroblasts by centrifugation at 250 g, 5 min, 20 °C
- Remove the supernatant and wash the cells with 5 ml FGM followed by centrifugation at 250 g, 5 min, 20 °C
- Remove the supernatant and resuspend the pellet in an appropriate volume of FGM (1-2 ml FGM is recommended when thawing 1 cryovial with fibroblasts)
- Determine cell viability and live cell number with trypan blue exclusion test in counting chamber
- Seed the cells at a density of approx. 5,000 cells/cm^2 in culture dish/flask (total amount of culture medium for 60 cm^2 dish is 9 ml FGM and cell suspension) and culture the cells at 37 °C in a humidified incubator with 5 % CO_2

E-mail: info@primacyt.de, Tel.: +49 (0)385-3993-600, Fax: +49 (0)385-3993-602

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3. Subculture of primary human fibroblasts

- Passage of the culture is recommended at a density of approx. 70-90 %
- Warm FGM and 0.9 % NaCl (or PBS) in 37 °C waterbath
- Thaw 1x Trypsin/EDTA solution (0.05 % Trypsin/ 0.02 % EDTA)
- Remove the medium and wash the cells twice the 0.9 % NaCl or PBS
- Remove NaCl or PBS and add 1 ml/60 cm^2 1x Trypsin/EDTA (= 20 µl/cm^2) and incubate for 5 min at 37 °C
- Dislodge the cells by tapping the dish/flask gently
- Stop the reaction with 5 ml/60 cm^2 FGM (100-200 µl/cm^2) and transfer the cell suspension into a conical tube
- Centrifuge at 250 g, 5 min, 20 °C
- Remove the supernatant, loosen the pellet before adding 5 ml FGM and repeat centrifugation (250 g, 5 min, 20 °C)
- Remove the supernatant and loosen the pellet before adding FGM (volume depends on the pellet size)
- Determine cell viability and live cell number with trypan blue exclusion test in counting chamber
- Seed the cells at a density of approx. 1,700 cells/cm^2 in culture dish/flask (total amount of culture medium for 60 cm^2 dish is 9 ml FGM and cell suspension) and culture the cells at 37 °C in a humidified incubator with 5 % CO_2
- Plated cell number should not be below 1,700 cells/cm^2 (this allows a passage time of 4-7 days)
- Change the medium every 2-3 days

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