PRIMACYT
Cell Culture Technology

Thawing and culture of primary fibroblasts

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Version 02

Recommended medium

- FGM: for thawing and culture of fibroblasts (adults and juvenile, various species)

1. After arrival of the cryopreserved/ plated cells in your laboratory

- Place the cryovial with frozen fibroblasts immediately into the gas phase of a 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 (at 37 °C, 5 % CO_2) until approx. 80 % confluency

2. Thawing of primary fibroblasts

- Use FGM as thawing medium
- Warm water bath and FGM to 37 °C
- Fill 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 water bath 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 ml FGM is recommended when thawing 1 cryovial with fibroblasts)
- Determine cell viability and live cell number with trypan blue exclusion test in a 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

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3. Subculture of primary fibroblasts

- Passage of the culture is recommended at a density of approx. 70-90 %
- Warm FGM and PBS in 37 °C water bath
- Thaw 1x Trypsin/ EDTA solution (0.05 % Trypsin/ 0.02 % EDTA)
- Remove the medium and wash the cells twice with PBS
- Remove PBS and add 1 ml/60 cm^2 1x Trypsin/EDTA (= approx. 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 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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