

2. Freeze medium: FBS with 6% DMSO

Culture procedure

Thawing of frozen cells

1. Thaw the frozen cryovial by gentle agitation in a 37 °C water bath in 1-2 minutes.
2. Remove the cryovial from the water bath as soon as the contents are thawed, and decontaminate by wiping with 70% ethanol.
3. Transfer the thawed cell suspension to a centrifuge tube containing 10 ml of Complete culture medium, centrifuge at 125 g for 5-7 minutes.
4. Remove the medium by aspiration, resuspend the cells with 10 ml of the Complete culture medium by gently pipetting up and down.
5. Transfer the cells to a 10 cm cell culture dish.
6. Place the cells in a 37°C incubator with 5% CO₂.

Sub-culturing

Volumes are given for a 10 cm cell culture dish. Increase or decrease the amount of dissociation medium needed proportionally.

1. Remove the medium by aspiration.
2. Briefly rinse the cell layer with 1xDPBS to remove all traces of serum that contains trypsin inhibitor.
3. Add 1 ml of Trypsin-EDTA (0.25%) solution to the dish and observe cells under an inverted microscope until cell layer is dispersed.
4. Add 4 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C with 5% CO₂.