

PROTOCOL FOR FREEZING AND THAWING MAMMALIAN CELLS

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I. Freezing down

1. Preparing freezing media: 10% DMSO complete medium i.e., 10ml DMSO + 80ml complete medium----filter store @ 4°C.
2. Trypsinize and transfer cells from a T-25 Flask (confluent) to a 15ml-tube.
3. Centrifuge it @ ½ top speed for 5 minutes.
4. Resuspend cell pellet in 5 ml freezing medium.
5. Distribute 1ml into each freezing vials.
6. Put the freezing vials into freezing box and freeze it @ -80°C.

II. Thawing

1. Prewarm complete medium @ 37°C water bath.
2. Fetch the target cell vial from -80°C and put it into 37°C water bath ***immediately***.
3. After melting (about 1 minute), add the cells into T-25 Flask which contains the prewarmed complete medium about 6ml.
4. Place the Flask into the incubator ***as soon as possible***.