Cells for Drug Development

Freezing/Thawing of the cells

Material

- Freezing medium (Cat.No. INS-SU-1004)
- PBS
- FBS
- Trypsin/EDTA (TE)
- 15 ml plastic tube
- Vials suitable for freezing in liquid nitrogen

Preparation

Freezing

- Grow the cells to 90 % confluence.
- Wash the cells with PBS.
- Trypsinize the cells with TE.
- Resuspend the cells with 5 ml of PBS, containing 2% FBS.
- Transfer cell suspension in 15 ml plastic tube.
- Spin-down cells @200g for 5 min.
- Aspirate supernatant.
- Resuspend cell pellet with freezing medium (cell concentration $1x10^6$ cells per ml).
- Transfer cell suspension in “freezing” vial.
- Place vials into Mr. Frosty or comparable devices (to slowly cool down vial).
- Place Mr. Frosty in -70°C overnight.
- After 24 h transfer vial from -70°C to liquid nitrogen tank for long term storage.

Thawing

- Pipette 4 ml medium in a 15 ml plastic tube.
- Quickly thaw vial in preheated water bath (@37°C).
- Transfer thawed cell suspension to 15 ml plastic tube containing 4 ml medium.
- Spin-down cells @200g for 5 min.
- Aspirate supernatant.
- Resuspend cell pellet with cultivation medium.
- Transfer cells in desired cultivation device (recommended is T25 flask or 6 well).

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