Jun 05, 2019

Mammalian Cell Culture: Freezing

Kenneth Schackart[1]

[1]University of Arizona

Working dx.doi.org/10.17504/protocols.io.3szgnf6

Kenneth Schackart

ABSTRACT

This protocol describes how to freeze cells that are being cultured in a tissue culture flask (T-75 or T-25).

GUIDELINES

- Gloves must be worn at all times.
- Perform all tasks within biosafety cabinet.
- Anything entering biosafety cabinet must be generously sprayed with 70% ethanol (even you).
- When finished, wipe biosafety cabinet with 70% ethanol, and UV for at least 15 minutes.

MATERIALS TEXT

- Cell culture media (at least 4 mL for T-75 or 1 mL for T-25)
- Fetal Bovine Serum (FBS)
- DPBS
- Trypsin-EDTA
- Dimethylsulfoxide (DMSO)
- 15 mL centrifuge tube
- Cyrovial(s)

Wash Cells

1 Remove media from flask.

2 Using serological pipet, add **4 ml** DPBS to flask [**1 ml** for T-25].

3 Using serological pipet, remove DPBS and dispose into waste beaker.

4 Repeat the above 2 steps, for a total of 2 washes.

Trypsinize

5 Add **4 ml** warmed Trypsin-EDTA to flask [**1 ml** for T-25].

6 Wait for cells to detach.

7 Add **4 ml** warmed cell culture media to flask to neutralize Trypsin-EDTA [**1 ml** for T-25]

Pellet Cells

8 Using a serological pipet, transfer cell suspension into 15 mL centrifuge tube.

9 Centrifuge cell suspension at **1500 rpm** for **00:03:00** .

Resuspend

10 Remove bulk of supernatant with serological pipet.

11 Remove remaining supernatant with 1000 µL pipette. For small cell pellets, it is best to leave a little supernatant to avoid disturbing the pellet.

12 Add **950 µl** of either FBS or cell culture media and allow to sit for **00:01:00** to make resuspension easier.

Some researchers prefer to use FBS as a freezing medium while others prefer whole media.

13 Gently pipette mix cell pellet until resuspended.

14 Add **50 µl** DMSO to cell suspension.

5% DMSO solution prevents ice crystals from forming in the liquid, minimizing cell rupture during freezing.

Freeze

15 Place cryovial in deep freezer cell vial container.

16 Allow at least **04:00:00** in deep freezer before transferring cryovial to liquid nitrogen tank.

This prevents too rapidly freezing, which may cause cell rupture.

Document

17 Update Lab Frozen Storage Inventory to reflect the new cell vial.