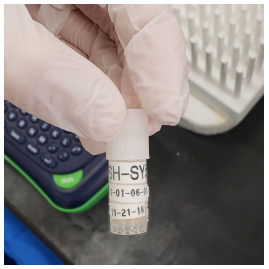
Jun 06, 2019

Mammalian Cell Culture: Freezing



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Protocol status: Working
We use this protocol and it's working

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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

- 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)

Before start

- Warm cell culture media, DPBS, and Trypsin-EDTA (and possibly FBS) in 37 °C water bath.
- Wash waste beaker with soap and warm water, then dry with paper towel.
- Expose serological pipet tips, centrifuge tube, and waste beaker to UV for at least 00:15:00 .
- Make label for cryovial with cell line, passage number, and date; affix to cryovial before UV.

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.

Note

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.

Note

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.

Note

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

Document

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