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Revival of Cells

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Protocol status: Working

We use this protocol and it's working

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Abstract

In cell biology, biotechnology, and medical research, cryopreserved cell resurrection is an essential procedure. To maintain cellular integrity, viability, and function, cryopreserved cells must be successfully thawed and recovered. In order to ensure effective recovery and minimal loss of cellular integrity, this protocol describes a standardized technique for cryopreserved cell revival. To minimize cellular damage and promote the best possible recovery, the technique includes crucial steps including controlled-rate thawing, suitable media selection, and post-thaw culture conditions. This technique can be used with a variety of cell types, such as immortalized cell lines, stem cells, and primary cells. This protocol attempts to increase reproducibility, reduce variability, and improve the overall success of cell culture experiments by standardizing the processes for cell revival.

Guidelines

Dilute the thawed cells slowly, using pre-warmed growth medium.

Materials

- Cryovial containing frozen cells
- Complete growth medium, pre-warmed to 37°C
- Disposable, sterile centrifuge tubes
- Water bath at 37°C
- 70% ethanol
- Tissue-culture treated flasks, plates, or dishes

Safety warnings

- ❗ Centrifugation to be done lightly to prevent damage to cells.



- 1 Remove the cryovial containing the frozen cells from liquid nitrogen storage and immediately place it into a  37 °C water bath.
- 2 Quickly thaw the cells (<  00:01:00) by gently swirling the vial in the  37 °C water bath until there is just a small bit of ice left in the vial.
- 3 Take the vial it into a laminar flow hood. Before opening, wipe the outside of the vial with 70% ethanol.
- 4 Transfer the thawed cells dropwise into the centrifuge tube containing the desired amount of pre-warmed complete growth medium appropriate for your cell line.
- 5 Centrifuge the cell suspension at approximately  200 x g for  00:10:00 . The actual centrifugation speed and duration varies depending on the cell type.
- 6 After the centrifugation, aseptically decant the supernatant without disturbing the cell pellet.
- 7 Gently resuspend the cells in complete growth medium, and transfer them into the appropriate culture vessel and into the recommended culture environment.