

Serum-Free Cell Freezing Medium

Introduction

Cell cryopreservation is an important process for preserving cells. The best technique for cell cryopreservation is storing them in liquid nitrogen with an appropriate freezing medium. Cryopreserved cells can be recovered for culture when needed.

Serum-Free Cell Freezing Medium is a sterile, serum-free medium, which is suitable for the cryopreservation of cells. This medium is ready-to-use, and does not need any additives, which is very easy to use.

This medium is chemically defined, containing nutrients such as glucose, amino acids, cryoprotectant dimethyl sulphoxide (DMSO) and phenol red. Meanwhile, this medium is FBS-free, which can reduce cryopreservation differentiation and cell contamination caused by serum batch.

Components and Storage

Components	K2820-50 mL	K2820-100 mL
Serum-Free Cell Freezing Medium	50 mL	100 mL
Store the product at 4°C, stable for 1 year.		

Protocol

1. Cell cryopreservation

- 1) Harvest cells to perform a cell count. Then according to the cell density, calculate the required volume of Serum-Free Cell Freezing Medium. The recommended cell density for cryopreservation is 1×10^6 - 1×10^7 cells/mL.

***Note:** Cryopreserved cells should be in the late logarithmic growth phase.

- 2) Centrifuge at 800 rpm for 5 min, and remove the supernatant.

***Note:** Centrifugation speed and time varies depending on the cell type.

- 3) Resuspend gently the cells in appropriate Serum-Free Cell Freezing Medium. The volume of Serum-Free Cell Freezing Medium varies depending on the cell type.
- 4) Aliquot the cell suspension into labeled sterile cryovials and tighten the cap of the cryovials.

***Note:** Always use sterile cryovials to store frozen cells. Do not use centrifugal tubes instead of cryovials. Centrifugal tubes don't

reliably seal and may fill with liquid nitrogen when frozen, leading them to burst on thawing.

- 5) Transfer the cryovials into a cell freezing container and immediately store the container at -80°C for a least 24 h. Then the frozen cells should be transferred to the vapor phase of liquid nitrogen for long-term storage.

***Note:** The cryovials in the cell freezing container should be transferred to -80°C as soon as possible.

2. Cell recovery

- 1) Remove cryovials from liquid nitrogen quickly and immediately place into a pre-warmed 37°C water bath for thawing.

***Note:** Keep the cap out of the water to reduce the risk of contamination.

- 2) When the contents are completely thawed, transfer the cell suspension into a centrifuge tube quickly. Add 5-10 mL pre-warmed, complete growth medium into the tube and mix gently.
- 3) Centrifuge at 800 rpm for 5 min, and remove the supernatant.

***Note:** Centrifugation speed and time varies depending on the cell type.

- 4) Resuspend gently the cells in appropriate pre-warmed, complete growth medium. Transfer the cell suspension into sterile shake flasks and place them in a shaker.

Note

1. The cryovials in the cell freezing container should be transferred to -80°C as soon as possible.
2. For your safety and health, please wear lab coats and gloves during the experiment.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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