BioResearch

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# Protocol for Cryopreservation of Nucleofection™ Competent Cells

Nucleofector™ PLUS Supplements can be used to cryopreserve ready-to-use primary cells or cell lines in Nucleofector™ Solution. For this purpose, the standard supplement must be replaced by Nucleofector™ PLUS Supplement when preparing the respective Nucleofector™ Solution. Once suspended in supplemented Nucleofector™ PLUS Solution cells can be stored in either the liquid nitrogen phase or in the gas phase. We recommend storage in the gas phase in general as liquid nitrogen may seep into the cryovial potentially causing the vial to "burst" upon thawing.

## Freezing Guidelines

**Note:**
Cells to be frozen should be free of contamination and in the appropriate phase of growth (see dedicated optimized Nucleofection™ Protocol for further details).

1. Grow the cell line or isolate the primary cell as described in the optimized Nucleofection™ Protocol.
2. **Replace** the standard supplement delivered with the Nucleofector™ Kit by the dedicated Nucleofector™ PLUS Supplement. To find out which Nucleofector™ PLUS Supplement is compatible to a certain Nucleofector™ Kit, please check the list at: **www.lonza.com/n-plus**.
3. Allow Nucleofector™ Solution and Nucleofector™ PLUS Supplement to equilibrate at room temperature for 15 minutes.
4. Add the Nucleofector™ PLUS Supplement to the Nucleofector™ Solution.
5. Count an aliquot of the cells and determine cell number.
6. Centrifuge the required number of cells as described in the optimized Nucleofection™ Protocol and carefully discard the supernatant.
7. Re-suspend the cell pellet carefully in appropriate volume of supplemented Nucleofector™ PLUS Solution (20 or 100 µl per planned Nucleofection™ Sample depending on Nucleofection™ Vessel to be used). Accomplish this step at room temperature.
8. Aliquot the cells in cryovials as quick as possible. The cells should not stay in Nucleofector™ PLUS Solution for more than **10** minutes prior freezing.

**Note:**
The size of the cryopreserved cell aliquots should be in accordance with the Nucleofection™ Experiments planned. It is recommended choosing cryovials of a size suited for the total volume of the cryopreserved cell aliquots (recommendation for minimum filling: 25% of the vial volume). Storage of small cell aliquots in huge cryovials bears the risk of water sublimating to the lid of the cryovial. Due to this phenomenon, the physical parameters of the cell aliquots will change over time which may lead to reduced performance of the cryopreserved cells.

9. Freeze the cell aliquots in a controlled manner by decreasing the temperature by 1°C per minute down to at least to -80°C (recommendation: use a manual device e.g. CoolCell®, Biocision for this purpose). The cells can be stored at -80°C for up to 24 hours prior to the transfer to the cryogenic storage. If you use a controlled rate freezer, decrease the temperature to at least below -125°C before you transfer the vials to the cryogenic storage.
10. Store the Nucleofection™ Competent Cells in the cryogenic storage until they will be used for a Nucleofection™ Experiment.

**Note:**
Nucleofection™ Competent Cells are stable for several months to years if stored properly. However, after one year of storage transfection performance may decrease. Therefore it is recommended to run a test transfection before using cells that were stored for a prolonged period of time (more than 12 months).

## Thawing Guidelines

11. Retrieve the cells from the cryogenic storage and transfer them immediately to a 37°C water bath. The cells must thaw quickly (**1 to 2 minutes**) as the temperature range between -50°C and 0°C bears the greatest risk for cellular damage. Thus, passing this temperature range as quickly as possible preserves viability of the competent cells.
12. Gently remove the cells from the vials and transfer them into the appropriate Nucleofection™ Vessels.
13. Mix the cells with substrate of choice by gentle pipetting.
14. Continue with the Nucleofection™ Process as described in the dedicated optimized protocol.

**Note:**
Nucleofection™ Competent Cells must be transfected as quickly as possible after thawing and addition of the substrate. The time window mentioned in the cell-type specific Nucleofection™ Protocol does **NOT** apply for cells cryopreserved in Nucleofector™ PLUS Solution. The viability will substantially decrease if thawed cells are kept at room temperature for more than **10** minutes after thawing.

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