

## Freezing cells - without freezing machine

Biochrom AG Information

The golden rule to freeze cells is: "The cells should cool down one degree (Celsius) per minute." (1, 2, 3, 4). In the following, we present a method to cool down cells according to the rule, if you haven't a programmable freezer machine available.

Use the new Biochrom freezing medium Biofreeze for the protection of the cells

### Required material:

- cells, absorbed in a freezing medium (e.g. Biofreeze) as a protection
- cryo tubes
- polystyrene box (thickness approx. 10 mm, closable)
- cellulose
- freezer
- liquid nitrogen

### Procedure:

1. Lay the polystyrene box out with cellulose and precool the polystyrene box in the freezer at -70 degrees (Celsius) for approximately 30 minutes.
2. Ideally, you additionally enwrap the prepared cryo tubes separately in cellulose.
3. Put the cryo tubes as separately as possible in the precooled polystyrene box.
4. Close the polystyrene box and put it into the freezer at -70 degrees (Celsius).
5. If you have followed the instructions, the material should cool down approximately one degree (Celsius) per minute. The polystyrene box should be stored at least for 2 hours (alternatively over night) at -70 degrees (Celsius).
6. If the cryo tubes have reached -70 degrees (Celsius), they are transferred into the box with liquid nitrogen. The transfer of the tube should be carried out as quickly as possible, as the cryo tubes warm up ten times quicker than they cooled down (1).



**Recommended number of cells according to bibliographical reference:**

- Suspension cells 5 x 10<sup>6</sup> Cells/ml (1)
- Adherent cells 1 x 10<sup>6</sup> Cells/ml (1)
- Very small cells 1 x 10<sup>7</sup> Cells/ml (1)
- 2 - 4 x 10<sup>6</sup> cells/ml, each 1 ml per cryo tube (2)
- disperse 5 x 10<sup>6</sup> - 2 x 10<sup>7</sup> cells/ml in 1- or 2 ml-cryovials (3)
- freeze 1 x 10<sup>6</sup> cells/ml in respectively 1 ml – portions (4)

**References:**

- 1: Leitfaden f. d. Zell- u. Gewebekultur, Hans Jürgen Boxberger – Wiley-VCH
- 2: Zukunftstechnologie Tissue Engineering, W .W. Minuth, R. Strehl, K. Schumacher – Wiley-VCH
- 3: Einfrieren und Auftauen von Zellen, IPS-2001-4255 – International Foundation Regenerative Medicine
- 4: Biochrom AG, F&E

**Further information regarding freeze medium Biofreeze:**

<http://www.biochrom.de/en/company/news/news-detail/article/neues-einfriermedium-der-biochrom-ag-mehr-sicherheit-fuer-saeugerzellen-mit-biofreeze/>