

## Convincing results from experience: Freezing with Biofreeze

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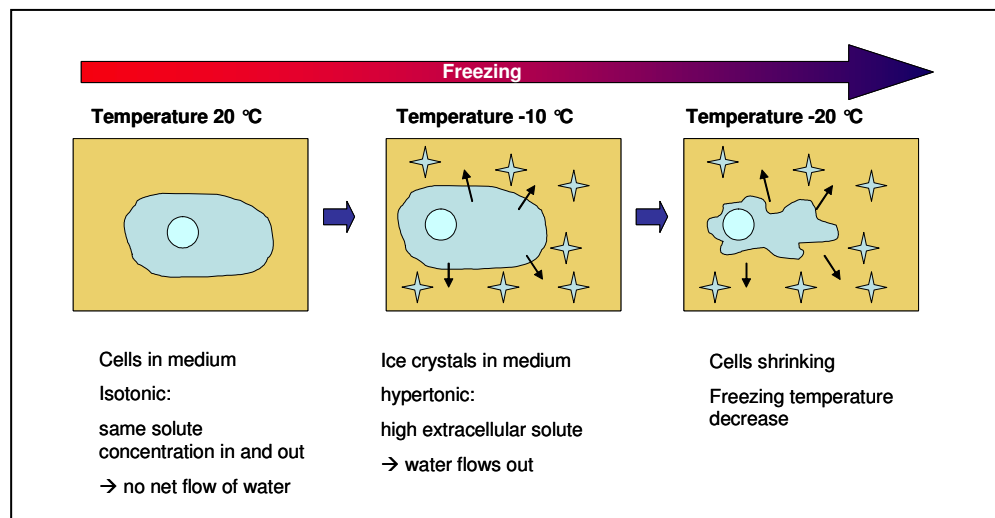
Biofreeze, manufactured by Biochrom AG, is a chemically defined serum- and DMSO-free freezing medium for the cryopreservation of cell cultures in fluid nitrogen. Biofreeze is intended for freezing and storing a variety of mammalian cell types and therefore replaces conventional cryopreservation media. Classical freezing media contain medium, serum and DMSO or glycerine. Verifiable, Biofreeze renounce serum as well as DMSO and additionally it contains no animal components.

Results from customers from experience show: Biofreeze is non-cytotoxic and the vitality of the cells after thawing is comparable with the vitality of cells that have been frozen in FBS and DMSO. Biofreeze can be used with all conventional freezing techniques.

### 1 Cryopreservation

The cryopreservation process is an exhausting procedure for each cell. Freezing causes ice crystals in the medium of the culture as well as in the cells themselves. To obviate these damaging ice crystals DMSO and glycerine is used. Additionally serum is inserted because of the protective effect of its components. Biofreeze deliberately does not use animal components and works with alternatives which achieve comparable results.

Fig. 1: Freezing process



### 2 Toxicity assay and results

This toxicity tests showed that Biofreeze in concentrations between 0-10 % is not toxic for mammalian cells. For these tests 3T6 murine fibroblasts cultured 48 h in DMEM/F-12 (1:1) + 10 % FBS and different concentrations of Biofreeze were used. Table 1 presents the current dilutions of Biofreeze and the cell vitality of murine 3T6 fibroblasts measured with MTT-assay. There are no differences between the vitality of the mouse

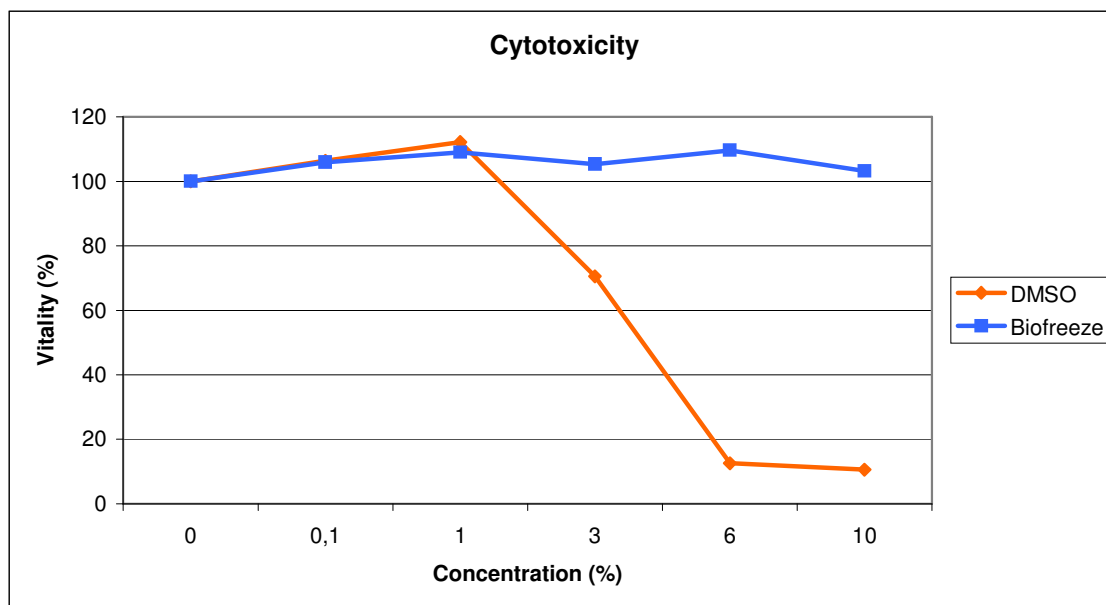
fibroblasts compared to the control cells found in this experiment (see fig. 2). In summary Biofreeze is not cytotoxic for mammalian cells.

**Tab. 1: Dilutions of Biofreeze and vitality of murine 3T6 fibroblasts after MTT-assay. Absorption is given as the arithmetic mean of three independent measurements.**

Dilution (%)	Biofreeze (µl)	Medium (µl)	Cell suspension (ml)	Absorption (570 nm)	Living cells/ ml after MTT
0	0	500	2	0.74	$3.3 \times 10^4$
0.1	2.5	497.5	2	0.77	$3.4 \times 10^4$
1	25	475	2	0.8	$3.5 \times 10^4$
3	75	425	2	0.77	$3.4 \times 10^4$
6	150	350	2	0.8	$3.6 \times 10^4$
10	250	250	2	0.76	$3.4 \times 10^4$

In comparison PAEC (porcine aortic endothelial cells) were incubated with ascending concentrations of DMSO. After 22 h at a concentration of 3 % DMSO a remarkable cytotoxic effect appeared. Between 6 and 10 % of DMSO the vitality of the porcine cell line decreased dramatically to 10 %.

**Fig. 2: Comparison of cytotoxicity of DMSO and Biofreeze in ascending concentration. 3T6 murine fibroblasts were incubated for 48 h in DMEM/F-12 (1:1) + 10 % FBS. In comparison PAEC (porcine aortic endothelial cells) were cultivated for 22 h with DMSO.**



### 3 Biofreeze by comparison

Biofreeze is the simple application. No dilution and no additional supplements are required. Re-suspend the cells directly in Biofreeze, transfer the cell suspension into plastic cryovials and place the cells into the freezer.

Thereby Biofreeze shows excellent results after freezing and thawing of various cells. Also sensitive cells like JURKAT, an immortalized T lymphocyte cell line, allows demonstrably storing cells with Biofreeze (Tab. 2).

**Tab. 2: Comparison between Biofreeze and conventional freezing media by using JURKAT cells.**

Parameters	Freezing medium			
	RPMI + FBS + DMSO	RPMI + FBS	Biofreeze	Biofreeze
Living cells/ml	$7.98 \times 10^5$	$8.31 \times 10^5$	$2.3 \times 10^6$	$4.47 \times 10^6$
Vitality (%) for freezing	100	98.6	97.8	97.4
Vitality (%) after thawing	95.9	7.6	97.1	93.0

The vitality of JURKAT cells after thawing with Biofreeze was 93 % at a cell density of  $4.47 \times 10^6$  and 97 % at a cell density of  $2.3 \times 10^6$ . Cells freezing with DMSO and FBS showed a similar vitality of 96 % after thawing. Verification of other cell lines like BHK-21, Vero, WISH and CHO caused in equal results. After thawing the cells demonstrated a good adherence, an excellent vitality as well as a good growth performance.

Therefore, Biofreeze is suitable for all mammalian cells which have to be cultivated without animal components, for example the development of pharmaceuticals or production of tissue replacements.

#### 4 Biofreeze details

Parameters	Biofreeze
Cat. No.	F 2270
Unit	25 ml
Storage temperature	+2 - +8 °C
Raw material	without DMSO, without Serum
Use	Freezing cells
Please note	for <i>in vitro</i> use



Order a free sample: [info@biochrom.de](mailto:info@biochrom.de)

How to use:

[http://www.biochrom.de/fileadmin/user\\_upload/service/Tipps\\_und\\_Hinweise/englisch/100519\\_hintergrundinformation\\_biofreeze\\_englisch.pdf](http://www.biochrom.de/fileadmin/user_upload/service/Tipps_und_Hinweise/englisch/100519_hintergrundinformation_biofreeze_englisch.pdf)

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