

## Datasheet

**Cryopan III****Chemically Defined and DMSO-free Cryopreservation Medium**

<b>Product</b>	<b>Description</b>	<b>Catalogue-No.</b>	<b>Size</b>
<b>Cryopan III</b>	<b>Chemically defined freezing medium, without DMSO</b>	<b>P07-96010</b>	<b>10 mL</b>
		<b>P07-96050</b>	<b>50 mL</b>
		<b>P07-96100</b>	<b>100 mL</b>
		<b>P07-96500</b>	<b>500 mL</b>

**Product description**

Cryopan III is a serum-free freezing medium without DMSO that is suitable for the cryopreservation of various cell types, including both primary cells and established cell lines. Its chemically defined composition, free from human or animal components, ensures optimal cell preservation. Cryopan III can be used for freezing adherent and suspension cells, making it a versatile option for the cryopreservation of animal and human cells across different cell types and culture conditions. The medium has also been proven effective for preserving tissue samples, e.g. umbilical cord tissue. The formulation without DMSO is particularly well-suited for primary cells and other applications in which the cytotoxic side-effects associated with DMSO are not desired.

**Storage conditions**

Storage conditions: -20 °C  
Stability: 2 years from date of production  
Filling: 10 ml, 50 ml, 100 ml, 500 ml, other sizes on request

**Composition**

Cryopan III consists of a chemically defined and optimized mixture of salts, sugar and additional antifreeze-substances. It contains no animal or human components and is free of DMSO.

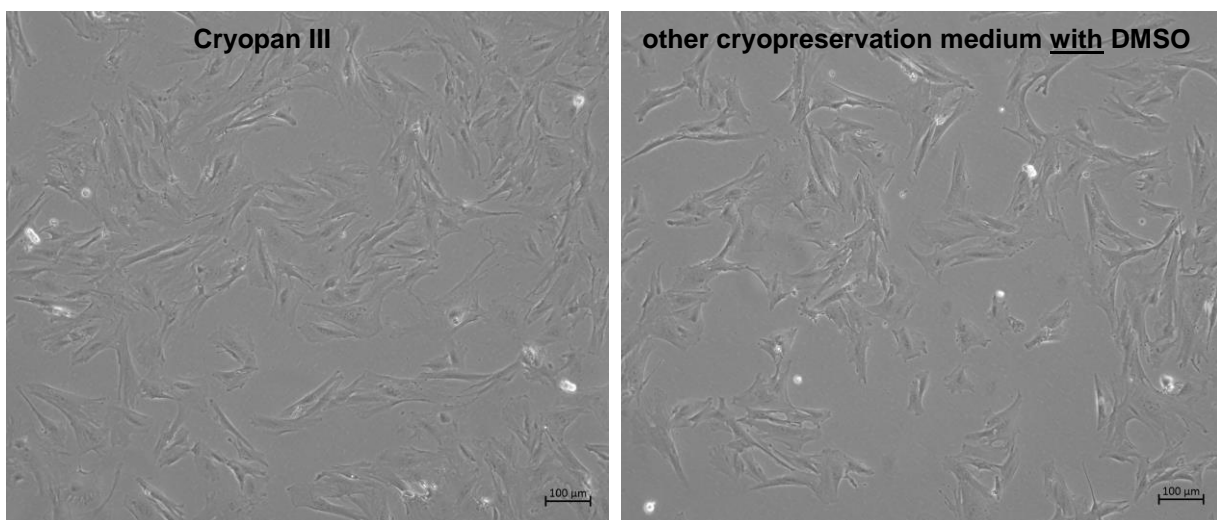
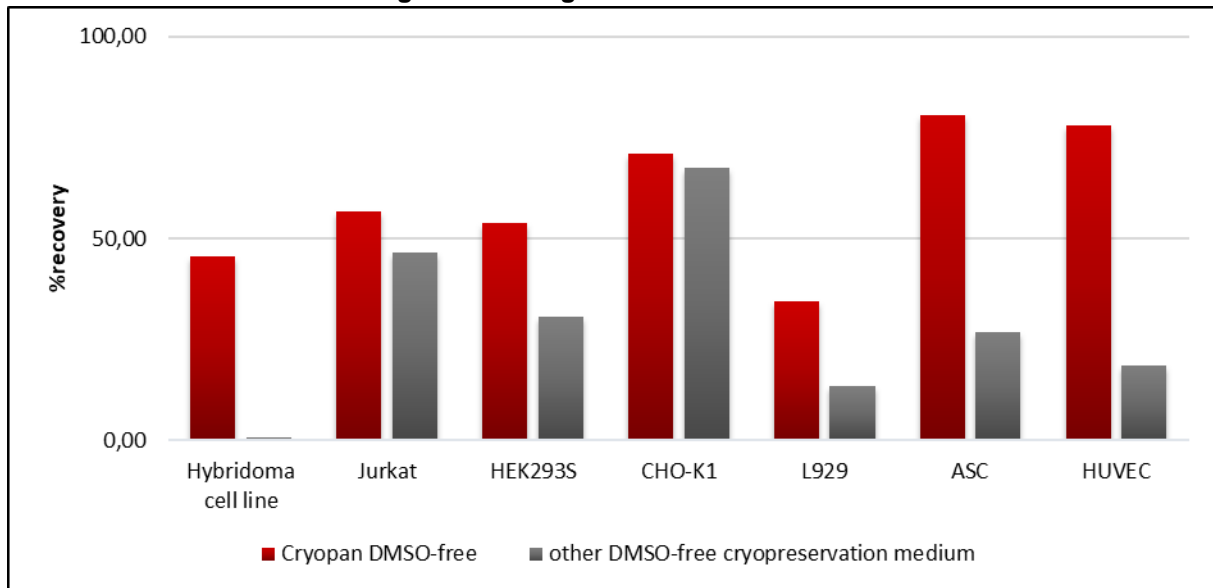
**Suitability**

Cryopan III is suitable for the cryopreservation of human and animal cell lines as well as primary cells.

**Special Advantages**

The serum-free formulation of Cryopan III is especially suitable for preserving serum-free cultured cells. The optimized composition of the product ensures a high level of cell viability following the thawing procedure compared to other available DMSO-free products.

### Survival Rate in % after freezing and thawing cells



**Primary MSC** in Cryopan III (left) and in other cryopreservation medium with DMSO (right) 3 days after thawing.

### Instructions for Use

Store the cryotubes in a cryotank filled with liquid nitrogen.

#### Freezing cells with Cryopan III

In order to achieve optimal results, it is recommended to use only viable cells in the logarithmic growth phase.

- Thaw Cryopan and store it at 2-8°C till using.
- For adherent cells, trypsinize and transfer them into the culture medium, followed by inactivation of trypsin activity using a trypsin inhibitor. Centrifuge the cells (100 - 200 g, 5 - 10 minutes).
- Discard the supernatant and wash the cell pellet in PBS (without Ca<sup>2+</sup>/Mg<sup>2+</sup>).
- After an additional centrifugation step, transfer the cells into PBS and determine the cell number and cell viability using trypan blue cell viability staining.

- Centrifuge the cells again and discard the supernatant.
- Transfer the cells into the cold Cryopan ( $5 \times 10^5$  -  $2 \times 10^6$  cells/ml Cryopan).
- Carefully suspend the cells by gently mixing the suspension through repeated pipetting until there are no more cell clumps.
- Refill the cell suspension into labelled cryotubes (0.5 – 1.5 ml per tube).
- Freeze the cells using an automatically or manually controlled cryo freezer. The optimal freezing rate is approximately 1°C per minute.
- Alternatively, place the tubes in a refrigerator for 15 minutes, allowing the freezing medium to penetrate into the cells. After this step, freeze the tubes at -20°C for 2 hours and then transfer them into the vapor phases of liquid nitrogen overnight.
- Store the cryotubes in a cryotank filled with liquid nitrogen.

#### Thawing cells

- Remove the cryotubes from the cryotank and promptly thaw them in warm water (not more than 2 minutes).
- Disinfect the exterior of the cryotubes using alcohol and, under sterile conditions, transfer the cells to a centrifuge tube.
- Add 10 ml of growth medium dropwise to the tube, ensuring careful mixing.
- Centrifuge the cells (150 - 200 g, 5 - 10 minutes).
- Discard the supernatant and resuspend the cells in the designated culture medium.
- Evaluate cell viability using a suitable methodology, such as FACS or trypan blue cell viability staining.
- Alternative: Due to the absence of cytotoxic DMSO, cells can be directly transferred into the prepared and pre-warmed culture medium by careful pipetting. For sensitive cell lines it is advisable to follow the prior thawing protocol.

#### **Technical support**

For technical support, questions or remarks please contact your local PAN-Biotech partner or the technical department of PAN-Biotech via email ([info@pan-biotech.com](mailto:info@pan-biotech.com)) or phone +49-8543-601630.

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