

(Cellular cryoprotectant)

Research use only

CP-1[®] High Grade

Cryoprotectant reagent for hematopoietic stem cells

For cryopreservation of hematopoietic stem cells, dimethyl sulfoxide (DMSO) has generally been used as a cryoprotectant.

In the case of cryopreservation with DMSO alone, it was common to be stored in liquid nitrogen (-196 °C) after frozen by the stepwise freezing method with a program freezer. However, from the early 1980's, simple cell freezing methods that do not use a program freezer have been studied using DMSO and hydroxyethyl starch (HES) for hematopoietic stem cells in bone marrow^{1), 2)}.

In Japan, studies on application of simple cell freezing method using DMSO and HES to peripheral blood stem cells have been advanced, and the optimum concentrations of HES and DMSO in the simple cell freezing method were decided 6% HES, 5% DMSO. This simple cell freezing method enabled stable cryopreservation for a short period of time (6 months to 1 year and a half) using a -80°C freezer without stepwise freezing method.³⁻⁸⁾

[Notes]

1. This product is a reagent for *in vitro* research, and use of this product for medical treatment has not been approved.
2. Please read this instruction carefully before using
3. Please be careful not to accidentally swallow this product. If you swallowed this product by mistake, please vomit as soon as possible. If this product contacts to your eye or skin, please wash out immediately. Please seek for medical treatment immediately if abnormal symptoms appear.

[Composition]

Composition (Per bottle)	For 100 mL	For 50 mL
HES (Hydroxyethyl starch) ¹⁻¹¹⁾	12 g	6 g
DMSO (Dimethyl sulfoxide) ¹⁻¹¹⁾	10 mL	5 mL
+ Isotonic sodium chloride solution ⁹⁾		
Total	68 mL	34 mL

[Intended use]

Protection of hematopoietic stem cells and other cells from freezing damage

[Caution on operation]

1. Please visually check there is no turbidity or floating matter before use.
2. Please perform all the procedures under sterile conditions.
3. Please use rapidly after addition of human serum albumin.
4. At the time of mixing this product with human serum albumin (HSA), and also, the mixture solution with cell suspension, heat may be generated. Therefore, please mix on ice.

[Method of use (for hematopoietic stem cells)]

1. Preparation method of CP-1[®] High Grade with HSA

Sterilize the rubber stopper of the vial of this product, with ethanol for disinfection, add HSA to the vial gradually and gently, and mix. The procedures may be accompanied by heat generation. Please perform on ice.

- (1) For 100 mL
Add 32 ml of 25% human serum albumin (HSA)* to make 100 ml.
- (2) For 50 mL
Add 16 ml of 25% human serum albumin (HSA)* to make 50 ml.

*25% human serum albumin can be substituted with 20% human serum albumin or autologous plasma. In any case, the same amount is added.

2. Preparation method of cell suspension

Please perform all the procedures under sterile conditions.

If necessary, wash the separated cells with phosphate buffered saline (PBS) and prepare the cell suspension by one of the following methods (1) and (2)^{7), 11)}. In addition, add anti-coagulant (Heparin, ACD solution, etc.) as appropriate.

- (1) In the case, Isotonic sodium chloride solution is used for cell suspension
Add Isotonic sodium chloride solution to the separated cells, and adjust the cell density to about 2×10^8 cells / mL.
- (2) In the case, RPMI 1640 medium is used for cell suspension
Add RPMI 1640 medium to the separated cells, and adjust the cell density to about 2×10^8 cells / mL.

3. Method of cryopreservation

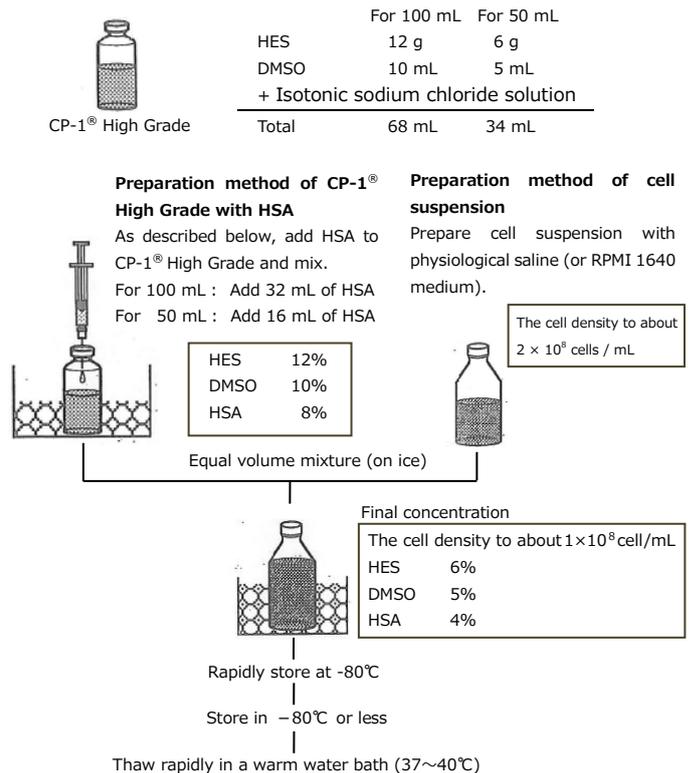
Please perform all the procedures under sterile conditions.

- 1) To the cell suspension, add an equal volume of CP-1[®] High Grade with HSA gently and gradually, and mix. Please mix them on ice. (When using a freezing bag, gently mix it while injecting CP-1[®] High Grade with HSA from the bag line into the cell suspension in the bag little by little with a syringe.)
- 2) Distribute the cell suspension to containers for cryopreservation rapidly, and store at -80°C. The target freezing rate is about -2 to -3 °C / minute.
- 3) After frozen, please keep as freezer at -80 °C or below (reported to be more stable when stored in liquid nitrogen after freezing in a -80°C freezer⁸⁾).

4. Thawing method

At the time of use, thaw the frozen cells rapidly in a warm water bath (37~40°C) (The target time for completion of thawing is 2-3 minutes).

[Flowchart] ⁹⁻¹¹⁾



[Precautions for handling and use]

1. Precautions for handling

The components of this product may show the following toxicities in humans on occasion.

HES has been pointed out the tissue deposit ability when entering the body. It has been reported that it may cause kidney and liver dysfunction, itching of the skin, allergic reactions, etc. (It is also pointed out in some reports that it may cause kidney damage.)^{12), 13)}

DMSO irritates the eyes and the skin, and in the case of high density may cause a lowering of consciousness. Inhalation may cause headaches and nausea. Ingestion may cause nausea, vomiting and lethargy. If it contacts with the eyes, it may cause redness and blurred vision.¹⁴⁾

Acute toxicity of DMSO¹⁴⁾

Animal species	Route	Fatal dose	Toxic dose etc.
Humans	Dermal	TDL ₀	1,800 mg/kg
Rats	Oral	LD ₅₀	14,500 mg/kg
Rats	Oral	LD ₅₀	17,400 mg/kg
Mice	Oral	LD ₅₀	7,920 mg/kg
Mice	Oral	TDL ₀	10.91 mL/kg
Guinea pigs	Oral	LDL ₀	> 11,000 mg/kg
Dogs	Oral	LD ₅₀	> 10,000 mg/kg
Rats	Inhalation	LCL ₀	> 1,400 mg/m ³ (4hr)
Rats	Dermal	LD ₅₀	40,000 mg/kg
Mice	Dermal	LD ₅₀	50,000 mg/kg

2. Precautions for use

- (1) Sterility of this produce is guaranteed before opening. Please use it promptly after opening.
- (2) Please strictly observe the storage condition.
- (3) Do not use if the bottle is found to be damaged, or if foreign matter is found in the container.
- (4) When this product is stored at a low temperature, some components may be deposited on occasion.
- (5) Please discard the residues after use as treated as infectious waste. Disposal should be in accordance with applicable laws and regulations.

* [Shelf Life]

- | | |
|-----------------------|---------------------------|
| (1) Storage condition | Store at room temperature |
| (2) Valid period | 12 months |

[Packaging]

Product Code	Product name	Packaging
551-27206-2	Cellular cryoprotectant	For 100mL×2
551-27207-9	CP-1®High Grade	For 50mL×2

[References]

- 1) Stiff PJ. et al. Unfractionated human marrow cell cryopreservation using dimethyl sulfoxide and hydroxyethyl starch. *Cryobiology*. 1983, vol.20, No.1, p. 17-24.
- 2) Stiff PJ. et al. Autologous bone marrow transplantation using unfractionated cells cryopreserved in dimethyl sulfoxide and hydroxyethyl starch without controlled-rate freezing. *Blood*. 1987, vol.70, No.4, p. 974-978.
- 3) Makino S. et al. Simple preservation method of bone marrow and peripheral stem cells by freezing. *Journal of Clinical and Experimental Medicine* Vol. 151, No.1, 1989. 10.7.

- 4) Makino S. et al. A simplified method for cryopreservation of peripheral blood stem cells at -80 degrees C without rate-controlled freezing. *Bone Marrow Transplantation*. 1991, vol.8, No.4, p. 239-244.
- 5) Takaue Y. et al. Comparative analysis of engraftment after cryopreservation of peripheral blood stem cell autografts by controlled- versus uncontrolled-rate methods. *Bone Marrow Transplantation*. 1994, vol.13, No.6, p. 801-804.
- 6) Sunami K. et al. Stem cell preservation by CP-1. *Low Temperature Medicine*. 1998, Vol.24, No.4, p. 171-174.
- 7) Hiramatsu Y. et al. Efficiency improvement of collecting and preserving peripheral blood stem cells. *Journal of the Japan Society of Blood Transfusion*. 2000, Vol.46, No.1, p. 1-6.
- 8) Makino S. Preserving of Peripheral blood stem cells. *Journal of Clinical and Experimental Medicine*. 1996, vol.176, No.9, p. 579-582.
- 9) Kishino K. et al. Study on the maintenance of hemopoietic stroma function of apheresis stem cells in the simple freeze preservation method. *Proceedings of Medical Technologists in Jichi Medical School Hospital*. 1992, No.16, p. 123-125.
- 10) Katayama Y. et al. The effects of a simplified method for cryopreservation and thawing procedures on peripheral blood stem cells. *Bone Marrow Transplantation*. 1997, vol.19, No.3, p. 283-287.
- 11) Makino S. et al. Efficacy and safety of cryoprotectant fluid CP-1 in peripheral blood stem cell transplantation. *Kyosei Medical Journal*. 2012, Vol.61, No.2, p. 223-226.
- 12) Takaori M. Plasma substitute HES. *Kokuseido*. 2010, p. 65-116.
- 13) Perner A. et al. Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. *The New England Journal of Medicine*. 2012, vol.367, No.2, p. 124-134.
- 14) Environmental Risk Assessment Office, Ministry of the Environment. Evaluation and management of chemicals. Vol.13. 1-2 (II) [6] Dimethyl sulfoxide. Ministry of the Environment.2015, p. 9.

[Contact]

Marketing & Scientific Affairs Div.
KYOKUTO PHARMACEUTICAL INDUSTRIAL CO., LTD.
7-8, Nihonbashi Kobuna-cho, Chuo-ku, Tokyo, 103-0024, JAPAN
Phone +81-3-5645-5664

manufactured by

製造販売元  極東製薬工業株式会社
茨城県高萩市上手綱朝山3333-26

本社住所 東京都中央区日本橋小舟町7-8