For cryopreservation of hemopoietic stem cells, dimethyl sulfoxide (DMSO) has generally been used as a cryoprotectant. However, with DMSO alone, after being frozen by the step-by-step freezing method with a program freezer, and stored in liquid nitrogen (-196°C), the recovery rate of stem cells was low occasionally. In addition, at the time of preservation for a long-term (more than 1 year), decrease in the recovery rate of stem cells was found similarly on occasion.

A mixed fluid of hydroxyethyl starch (HES) and DMSO is used for this product “CP-1” as a method of cryopreservation of stem cells of the bone marrow, peripheral blood and cord blood. By use of this product, stable cryopreservation of cells even for a long time is possible.

Furthermore, for short-term storage (6 months – 1 year), “CP-1” is even possible to preserve stem cells at -80°C without application of the step-by-step freezing method (Makino’s method). 7)

**[Composition]**

<table>
<thead>
<tr>
<th></th>
<th>For 100 ml</th>
<th>For 50 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyethyl starch (HES)</td>
<td>12 g</td>
<td>6 g</td>
</tr>
<tr>
<td>Dimethyl sulfoxide (DMSO)</td>
<td>10 ml</td>
<td>5 ml</td>
</tr>
<tr>
<td>Isotonic sodium chloride solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>68 ml</td>
<td>34 ml</td>
</tr>
</tbody>
</table>

At the time of use: To this product for 100 ml, add 32 ml of 25% human serum albumin to make 100 ml. To this product for 50 ml, add 16 ml of 25% human serum albumin to make 50 ml. (20% human serum albumin or self-plasma can also be used as substitute.)

**Precautions for use**

1. This product is a reagent for *in vitro* research, and use of this product for medical treatment has not been approved.

2. The components of this product may show the following toxicities in humans on occasion. If you swallowed this product by mistake, please vomit as soon as possible. If this product attached to your eye or skin, please wash out immediately. If any abnormality was found, please consult with a physician as soon as possible.

**Toxicity of HES**

The toxicity of HES to humans is very low, but if it enters into human body, the following symptoms may develop. Vomiting, fever, chill, itching, swelling of the submandibular and parotid glands, mild influenza-like symptoms, headache, myalgia, peripheral edema of lower limbs, various anaphylactoid reactions (edema around the orbit, urticaria, asthmatoed wheezing), hemodilution, enhanced blood stream, and hemorrhage due to pulmonary arterial edema. (Physicians’ desk reference (1992) pp. 948-950)
Toxicity of DMSO

Although the toxicity of DMSO to humans is low, it shows the following irritativeness (corrosiveness) to the eyes and skin. It is permeable to the skin, and by continuous contact with it, the skin becomes red due to dermal absorption, scaly shedding of skin may occur, and on occasion, nausea, vomiting, chill, convulsions, visual impairment or allergic action may develop. In addition, it has been reported that in experiments in animals such as dogs, rabbits and pigs, subcutaneous administration of DMSO induced cataract.

(Guide to Industrial Toxicity, Ishiyaku Publishers, Inc.)

Acute toxicity of DMSO:

<table>
<thead>
<tr>
<th>Route</th>
<th>Species</th>
<th>LD50</th>
<th>TDL0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>Rats</td>
<td>20 mg/kg</td>
<td>5 g/kg (Day 6-Day 12 of gestation)</td>
</tr>
<tr>
<td>Intravenous</td>
<td>Dogs</td>
<td>2.5 g/kg</td>
<td>8 g/kg (Day 6-Day 12 of gestation)</td>
</tr>
<tr>
<td>Oral</td>
<td>Rats</td>
<td>5 g/kg (Day 6-Day 12 of gestation)</td>
<td>5 g/kg (Day 6-Day 12 of gestation)</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>Mice</td>
<td>5 g/kg (Day 6-Day 12 of gestation)</td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td>Hamsters</td>
<td>50 mg/kg (Day 8 of gestation)</td>
<td></td>
</tr>
</tbody>
</table>

3. For disposal of this product, after autoclaving at 121°C for 30 minutes, please treat appropriately and discard.
4. Sterility of this produce is guaranteed before opening. After being opened, the sterility cannot be guaranteed.
5. When this product is stored by the method other than that in the description of the virtues of this product, the quality may not always be guaranteed for the entire guarantee period.
6. When the container is found broken, please don’t use and make contact with our company immediately.

【Purpose of use】
Protection of cells from freezing damage

【Characteristics】
• In comparison with the use of DMSO alone, long-term stable preservation of cells is possible.
• For short-term storage, it is possible to preserve at -80°C without application of the step-by-step freezing method when this product is used (Makino’s method).

【Method of use】
Please perform all the procedures under sterile conditions. The procedures 3 and 4 may be accompanied by heat generation. Please perform at low temperature (in ice-water bath).

<Step-by-step freezing method by use of a program freezer³1>
1. After washing the isolated cells with PBS 2 or 3 times, suspend the cells in RPMI 1640 medium, and adjust the cell density to 2-10 x 10⁷ cells/ml.
2. Suck 25% human serum albumin solution with an injection syringe; 32 ml for a 100-ml vial and 16 ml for a 50-ml vial.
3. Sterilize the rubber stopper of the vial of this product, “CP-1”, with ethanol for disinfection, add the albumin solution in 2 above to the vial gradually and gently, and mix.
4. To the cell suspension prepared in 1, add approximately an equal volume of this product to which human serum albumin has been added in 2 gently and gradually, and mix.
   (When a pack for storage is used, connect the connecting needle of the pack for storage to the rubber stopper of the vial of this product to which human serum albumin has been added in 2, and pour this product gradually to the
cell suspension in the pack for storage while gentle stirring.)
5. Distribute the cell suspension to containers for cryopreservation rapidly.
6. With a program freezer, chill the cell suspension at a freezing rate of -1 to -2°C per minute down to -40°C. (It was reported that freezing at a rate faster than -5°C/min decreased the cell recovery rate\(^5\).)
7. Furthermore, chill at a freezing rate of -10°C/min down to -90°C.
8. When the temperature decreased to about -90°C, put it into liquid nitrogen and store there.
9. At the time of use, thaw the frozen cells rapidly in a constant temperature bath at 37-40°C. (The target time for completion of thawing is 2-3 minutes.)

<Makino’s method>
The procedures from 1 to 4 are the same as those in <Step-by-step freezing method by use of a program freezer>
5. Distribute the cell suspension to containers for cryopreservation rapidly, and store at -80°C (there is a report stating that stable storage can be achieved in liquid nitrogen\(^3\)). The target freezing rate is about -2°C/minute.
6. At the time of use, thaw the frozen cells rapidly in a constant temperature bath at 37-40°C (the target time for completion of thawing is 2-3 minutes).

[Precautions for handling]
1. Since this product has been packed under sterile conditions, you can use as it is.
2. Please use rapidly after addition of human serum albumin.
3. At the time of mixing this product with 25% human serum albumin, and also, the mixture solution with cell suspension, heat will be generated. Therefore, please mix them at a low temperature without fail.
4. When this product is stored at a low temperature, some components may be deposited on occasion. Please store at room temperature.
For 100 ml 50 ml
HES 12 g  6 g
DMSO 10 ml 5 ml
Isotonic sodium chloride solution
Total 68 ml 34 ml

Add 32 ml of 25% serum albumin for 100 ml to make a total volume of 100 ml.
Add 16 ml for 50 ml to make a total volume of 50 ml.

12%-HES
10%-DMSO
8%-human serum albumin

Equi-volume mixture
(gentle mixing at low temperature (in ice water bath))

Cells, 1-5 x 10⁷/ml
HES 6%
DMSO 5%
Human serum albumin 4%
(gentle mixing at low temperature (in ice water bath))

Container for cryopreservation

<Step-by-step freezing method by use of a program freezer>

With a program freezer, chill the cell suspension at a freezing rate of -1 to -2°C per minute down to -40°C.
Furthermore, chill at a freezing rate of -10°C /min down to -90°C.
Store in liquid nitrogen (-196°C).

Thaw rapidly in a constant temperature bath at 37-40°C.

<Makino's method>

Rapidly store at -80°C.
Thaw the frozen cells rapidly in a constant temperature bath at 37-40°C.
【Storing method】
Store at room temperature

【Shelf life】
Six months

【Packaging】
• For 100 ml (Product Code No. 27200)
  68 ml x 6 vials
• For 50 ml (Product Code No. 27202)
  34 ml x 6 vials

【References】
2) S. Makino, Peripheral blood stem cells at -80°C without rate-controlled freezing.: Bone Marrow Transplantation 8(4)1991.
4) S. Makino: Method of preservation of peripheral stem cells. Journal of Clinical and Experimental Medicine 176 No.9 1996. 3.2.
5) N. Taguchi, T. Takahashi and S. Sekiguchi: Effect of freezing speed on preservation of peripheral stem cells by freezing. The 2nd symposium of Japan Society for Low Temperature Medicine 1-8, 1995. 11.17
6) Y. Kawano and J. Takagami: Hemopoiesis-reconstructing function in transplantation of peripheral stem cells after preservation by the simple freezing method. The 2nd symposium of Japan Society for Low Temperature Medicine 1-10, 1995. 11.17
7) Y. Takaue, T. Abe, Y. Kawano, et al.: Comparative analysis of engraftment after cryopreservation of peripheral blood Stem cell autografts by Controlled versus uncontrolled -rate methods.: Bone Marrow Transplantation 13(6)1994

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