RESEARCH USE ONLY

BioVerde Cell Cryopreserving Medium

Cryopreserving Medium for Various Cell Cultures

CryoScarless DMSO-Free

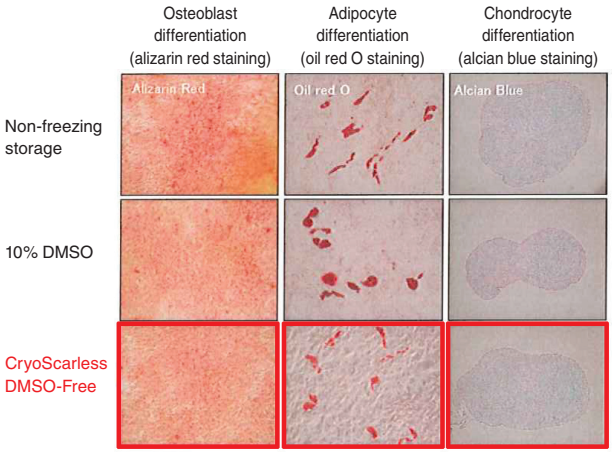
CryoScarless DMSO-Free is a serum-free and dimethyl sulfoxide (DMSO)-free medium for long-term storage at -80℃ or in liquid nitrogen.

Features

- Serum-free and DMSO-free formulation.
- High cell viability, and no risk of both cytotoxicity of DMSO and contamination by serum-derived proteins.
- Consistent and high cell viability after thawing (> 90% for most of the cell lines).
- Maintaining stem cell pluripotency after thawing.
- Free of bacteria, fungi and mycoplasma contamination.
- Long shelf life. The product is stable for 2 years at 4℃ after the date of manufacture.

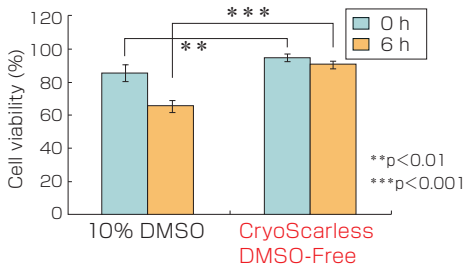
※ For Research Use Only. Not for Diagnostic Use.

Comparison Data



Differentiation of rat mesenchymal stem cells after thawing.

The results showed that cell pluripotency was maintained after thawing in rat mesenchymal stem cells cryopreserved in CryoScarless DMSO-Free.



Cell viability of rat mesenchymal stem cells after thawing.

Cell viability after cryopreservation with CryoScarless DMSO-Free was significantly higher than cells cryopreserved in 10% DMSO.
0 h : immediately after thawing, 6 h : after cell adhesion

Application

Cell type	Cell viability (%)
L929	97.5 ± 1.2
MG63	93.1 ± 2.3
HT1080	90.2 ± 4.3
Colon26	92.3 ± 2.3
B16F1	94.2 ± 0.6
KB	91.8 ± 0.9
Caco2	93.7 ± 1.9
MC3T3	94.4 ± 0.5
Jurkat E6-1	88.4 ± 2.5
HUVEC	89.9 ± 0.4
HCAEC	90.1 ± 1.6
MEF	94.4 ± 0.8
hACh	93.5 ± 0.7

※ Cells were cryopreserved in CryoScarless DMSO-Free for 3 months at -80℃ and these viability were measured at 24h after thawing.

Protocol Outline

1. Precipitate cultured cells by centrifugation and remove the supernatant.
2. Suspend the cells with CryoScarless DMSO-Free medium (1 ml for 5×10^5 - 10^6 cells). Dispense the cell suspension in 1 ml aliquots to cryopreservation vials.
3. Transfer the vials to -80℃ deep freezer.
   ※ For longe-term cryopreservation, transfer the vials to liquid nitrogen storage tank.
4. Remove the cryopreserved cell from storage and quickly thaw in a 37℃ shaking water bath. Immediately dilute and gently mix each 1 ml of cells with 10 ml of appropriate cell culture medium.

Ordering Information

Product Name Maker	Code	Size
CryoScarless DMSO-Free		
BVD	CPL-A1	100 ml

Funakoshi

Cryopreserving medium for ES and iPS cells

StemCell Keep

StemCell Keep is a highly efficient cryopreserving solution by vitrification for primate ES and iPS cells.

Features

- Animal-derived Protein-free and DMSO-free formulation. No risk of differentiation effect by DMSO.
- Maintain ES and iPS cells with colony formation by vitrification.
- Maintain stem cell pluripotency after thawing.
- High cytoprotection effect and vitrification technology by new original cryoprotectant material is allowed to cryopreserve ES and iPS cell colonies.
- Sufficient for 100 vials.
- Free of bacteria, fungi and mycoplasma contamination.
- Long shelf life. The product is stable for 2 years at 4℃ after the date of manufacture.

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Cryopreservation by Vitrification

Primate ES and iPS cells are quite sensitive to freezing damage.

- Primate ES and iPS cells had poor viability after slow-freezing at -80℃ in 10% DMSO.
- Colony of Primate ES and iPS cells had very poor viability under the same conditions stated above.

Cryopreservation of ES and iPS cells by vitrification.

- Vitrification is the transformation of a liquid into a glass.
- It is achieved by suspending the cells with vitrification solution and transfer it to liquid nitrogen chamber to prevent water transition.
- Cells can be protected from freezing damage.

Problems of conventional vitrification method for preservation of ES and iPS cells.

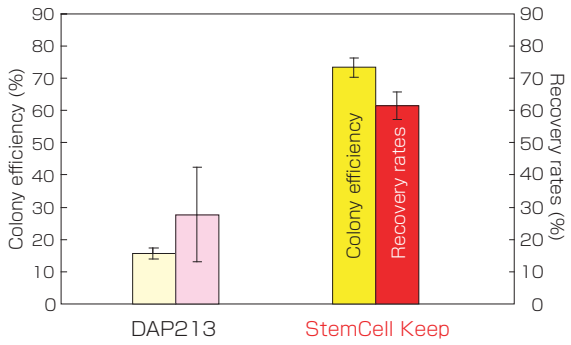
- Because vitrification occurred at a high solute concentration, cytotoxicity by high osmolarity is observed.
- Need for rapid thawing to prevent recrystallization.
- Need for experienced operators.

Problems of conventional vitrification solution

- Large volume (200 µl) of vitrification solution bring instability of vitrification conditions.
- DMSO and acetamide are included in conventional vitrification solution. DMSO has influence on OCT-4 expression, and acetamide is identified as carcinogens.

StemCell Keep is released as novel vitrification solution to improve conventional way.

Comparison Data



Colony efficiency and recovery rates of human iPS cells after thawing.

Data show the attached colony and recovery rates of human iPS cells after thawing. At 1 day after seeding, only 15% colonies were attached after cryopreservation with DAP213 (conventionally used as vitrification solution) compared to non-freezing storage control cells (100%). In contrast, 80% of the colonies were attached with StemCell Keep. At 4 day, cells had grown and formed large colonies; these were counted to evaluate recovery rates. Recovery date of iPS cells cryopreserved in StemCell Keep was up to 62%, which was singnificantly different from 28% of DAP213.

Ordering Information

Product Name Maker	Code	Size
StemCell Keep		
BVD	VPL-A1	20 ml

Protocol Outline

※ The preliminary experiment is necessary before the actual experiment is carried out.

Cryopreserving

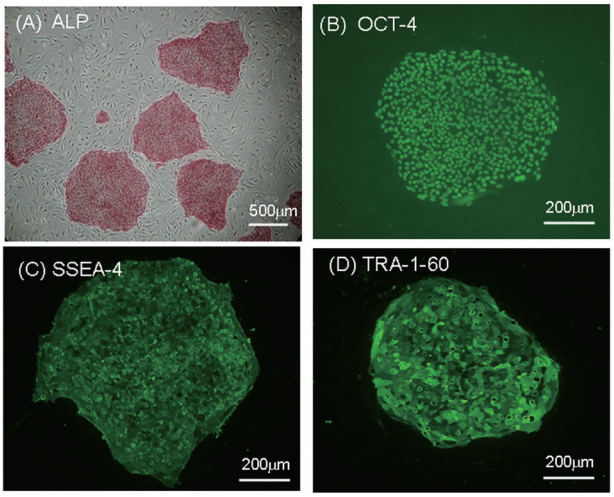
1. Place a dewar flask filled with liquid nitrogen in the clean bench.
2. Detach ES / iPS cell colonies with 0.25% trypsin / 1 mg/ml collagenase IV / in PBS.
   ※ One 60 mm dish of nearly confluent cells can be split into 1 - 5 vials.
3. Precipitate these cells by centrifugation and remove the supernatant. If several vials are needed for cryopreservation, they are stored in ice.
4. Suspend the cells with 200 µl of StemCell Keep by pipetting. Close a lid of the cryoreservation vial, and transfer it to the dewar flask within 1 minute.
   ※ If the vitrification is successful, the solution should be remained transparent.
5. Transfer the vial to the the liquid nitrogen chamber or -130℃ deep freezer.

Thawing

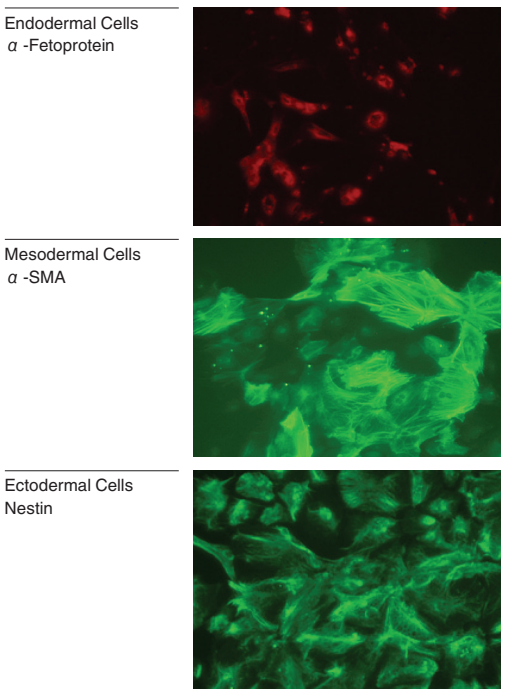
1. Prepare each centrifugation vials each containing 9 ml of cell culture medium warmed at 37℃ in water bath.
   ※ Thaw one tube at a time. Leave others frozen.
2. Put one cryopreservation vial containing cells into a dewar flask filled with liquid nitrogen, and place it on clean bench.
3. Add 1 ml of warmed cell culture medium into the cryopreserved vial immediately, and then mix gently by pipetting.
4. Transfer the entire volume of diluted cells into the centrifugation vial, and centrifuge to wash cells.
5. After transfer the cells to the feeder plate, continue the further culture procedures according to standard protocols.

Precausions Please prepare the medium and instruments for each experiment by reading the instructions in advance.

Experimental Results



Differentiation of human iPS cells after cryopreservation with StemCell Keep.
Cells are positive for all of undifferentiation markers, Alkaline phosphatase (A), OCT-4 (B), SSEA-4 (C) and TRA-1-60 (D), and are maintained in the undifferentiated state.



Pluriopotency of human iPS cells after cryopreservation with StemCell Keep.
After preparation of embryoid bodies from human iPS cells, the cells are kept for up to 1 week in normal culture plate. Each of differentiation marker is detected.

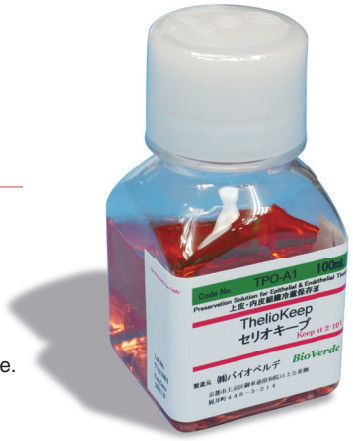
RESEARCH USE ONLY

BioVerde Tissue Preserving Medium

Preserving Medium for Dermal Tissue

ThelioKeep

ThelioKeep is a serum-free and dimethyl sulfoxide (DMSO)-free medium for preserving for 1 - 2 weeks at 4℃ .



Features

- Maintain the morphology and proliferative ability of epithelium and endothelium tissues.
- Tissues kept in ThelioKeep can be used for various applications.
- Serum-free and protein-free formulation.
- Free of bacteria, fungi and mycoplasma contamination.
- Long shelf life. The product is stable for 1 years at 4℃ after the date of manufacture.

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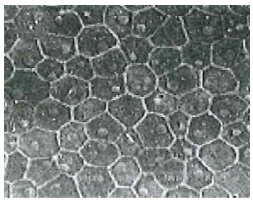
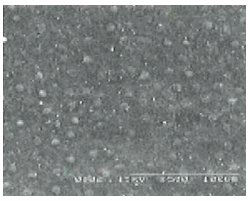
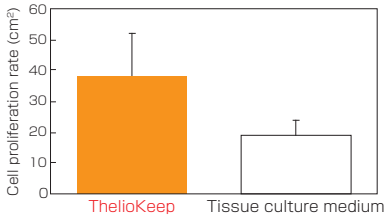
Comparison Data



ThelioKeep



Tissue culture medium



Comparison of the morphology and proliferative ability of corneal endothelium tissue.

Human cornal endothelium were preserved in ThelioKeep for 2 weeks at 4℃ .

Protocol Outline

1. Dispense 0.5 ml of ThelioKeep and put it into the microtube containing EGCG tablet provided. Pipette the entire solution back into the bottle of ThelioKeep and mix well.
2. After the EGCG is completely dissolved , transfer a few milliliters of solution to a centrifugation vial. Keep it cool at 4 - 10℃ .
   ※ Prepared ThelioKeep must be used as soon as possible. If storing for 1 - 2 weeks, separate into aliquot and keep at -20℃ . To prevent from loss of the activity, frequent freeze-thaw method should be avoided.
3. Immerse the tissue isolated in the centrifugation vial with closed lid. Store at 4℃ .
4. Rince the living tissue with PBS, and then transfer it to the tissue culture medium. Continue the further procedures according to appropriate protocols.
   ※ For tissue culture procedures, place them under physiological conditions as soon as possible.
   ※ For preparation of tissue sections, to preserve tissue morphology and retain the antigenicity of the target molecules, fix the tissue with appropriate fixation solution immediately.
   ※ For extraction of intracellular materials, wash the tissue with cooled PBS thoroughly.

Ordering Information

Product Name Maker	Code	Size
ThelioKeep		
BVD	TPO-A1	100 ml

NOTE ※ NOT FOR HUMAN USE. RESEARCH USE ONLY. NOT FOR DIAGNOSTIC, THERAPEUTIC OR COMMERCIAL USES.

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ISO 14001 JQA-EM5844

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Z1-U03T-01 (2011.03)