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BioVerde

Cryopreservation media for ES/iPS cells by Vitrification

DMSO/Serum/Protein/Xeno-free

StemCell Keep

For more information : http://www.funakoshi.co.jp/exports_contents/46011

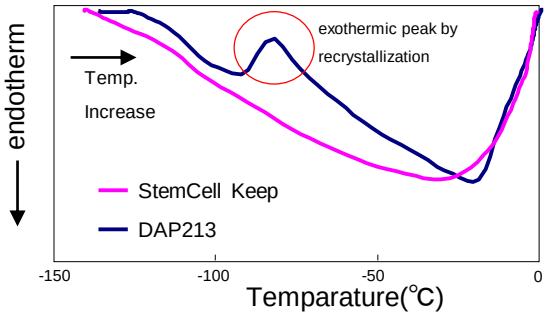
DMSO-free | Serum-free | Protein-free | Xeno-free

StemCell Keep is optimized cryopreservation media for primates ES/iPS cells by keeping high vitrification ability with low cyto-toxicity.

MEMO

Existent problem of preservation media contains DMSO and What is vitrification?

Very old method of cryopreservation was “Slow Programmed Freezing” using by 10% DMSO contained solution. However, big ice crystal is formed by this method and this crystal causes very poor cell viability (0.1-1%) for primate ES and iPS cells. Improved method is “vitrification” using by DAP213 (DMSO 2M, Acetamide 1M, Propylene glycol 3M).
Vitrification is the new method for preventing ice crystal formation by rapid freezing (<15 sec) in LN2. However, this method requires rapid thawing to prevent recrystallization and experienced operators. It is known that DMSO has influence on OCT-4 expression and differentiation, Acetamide is identified as carcinogens. Moreover, DAP213 is high osmolarity solution – it means this solution has high toxicity. Therefore, development of low Toxicity, easy to use vitrification solution had been expected. StemCell Keep is the solution that overcomes these disadvantages of DAP213!



Solution freezed by Liquid Nitrogen is heated by 50℃/min.

StemCell Keep does not have recrystallization during temperature rising.

Features

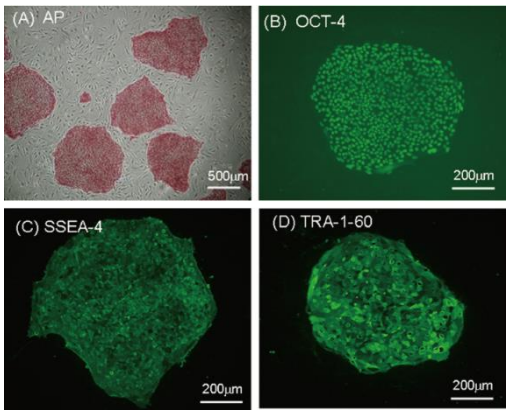
- Animal-derived Protein-free and DMSO-free formulation. No risk of differentiation effect by DMSO.
- Maintain ES and iPS cells with colony formation by vitrification.
- Maintain stem cell pluripotency after thawing.
- High cyto-protection effect and vitrification technology by new original cryoprotectant 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.

Trial Sample available!

Small size trial sample (5 mL) is available. Please contact your local distributors.

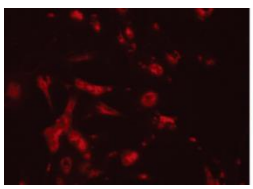
Experimental results and protocol are on next page.

Experimental Results

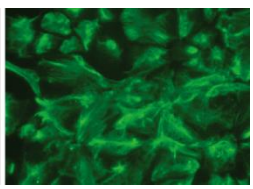


Differentiation of human iPS cells after cryopreservation with StemCell Keep.

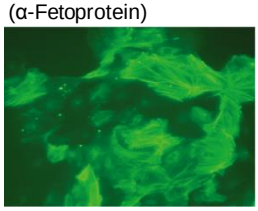
Cells are positive for all of undifferentiation markers, Alkaline phosphatase (A), OCT-4 (B), SSEA-4 (C) and TRA-1-60 (D), and are maintained in the undifferentiated state.



Endodermal Cells (α-Fetoprotein)



Ectodermal Cells (Nestin)



Mesodermal Cells (α-SMA)

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.

Protocol Outline

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

Cryopreserving

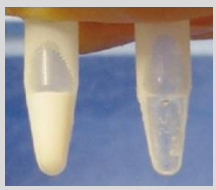
Handling procedure video is available from :
http://www.funakoshi.co.jp/exports_contents/46011

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 cryopreservation vial, and transfer it to the dewar flask within 1 minute.

※ If the vitrification is successful, the solution remains transparent.



Right : Vitrification is successful. (Successful Example)
Left : Recrystallization happened (Failed Example)

5. Transfer the vial to the liquid nitrogen tank 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.

Product Information

[Manufacturer : BVD]

Product Name	Size	Catalog #	Storage
StemCell Keep	20 mL	VPL-A1	4℃

NOTE

※ All products here are research use only, not for diagnostic use.
※ Specs might be changed for improvement without notice.
※ Company name and product name are trademark or registered mark.
※ Please contact your local distributors for orders, quote request and inquiry.

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