nacalai tesque
The quality for certainty.

Cell Reservoir One (Vitrify)

Vitrification has become an important alternative to standard slow programmable freezing methods for cryopreservation of primate ES cell lines including Human iPS cells because of the higher survival rates of cells after thawing. However, the vitrification requires an ultra-rapid freezing protocol, usually less than 15 seconds between making cell suspensions and freezing in liquid nitrogen.

Cell Reservoir One (Vitrify) is a novel serum-free cell culture freezing medium for vitrification method, which contains a water-soluble glycoprotein SERICIN isolated from the silkworm cocoon as a major constituent. It provides high survival rates of primate cells, such as Monkey ES cells and Human iPS cells even with a longer freezing protocol; up to 60 second from the cell collection to freezing in liquid nitrogen.

*Cell Reservoir One (Vitrify) is produced in corporation with SEIREN. (Patent pending)

Features

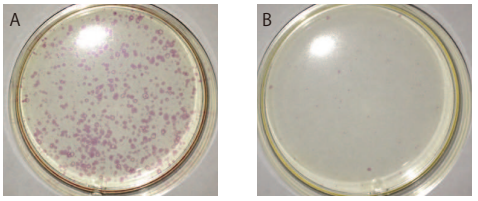
- *High viability with a longer freezing protocol (up to 60 seconds)*
- *Low toxicity to cells (DMSO and acetamide free)*

Application 1

Comparison of survival rate of Hunan iPS cells (201B7 cell line*)

*Takahashi, K. *et al. Cell,* 2007 Nov 30;**131**(5):861-872

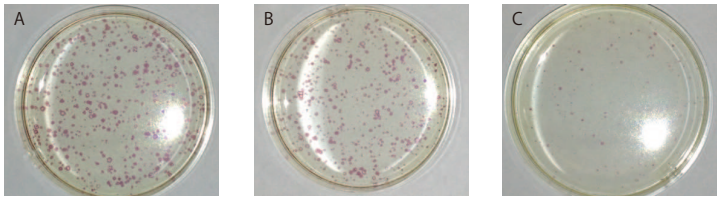
- **Freezing protocol:** 60 seconds



A: Cell Reservoir One (Vitrify)
B: DAP213

Human iPS cells were cryopreserved for more than 2 weeks in Cell Reservoir One (Vitrify) or DAP213. Viability was detected using Alkaline Phosphatase 4 days after thawing. Cell Reservoir One (Vitrify) showed high survival rate, while most of cells in DAP213 were dead.

- **Freezing protocol:** 15 seconds



A: Cell Reservoir One (Vitrify)
B: DAP213
C: Company A

Human iPS cells were cryopreserved for more than 2 weeks in Cell Reservoir One (Vitrify), DAP213 or Company A's product. Viability was detected using Alkaline Phosphatase 4 days after thawing. Cell Reservoir One (Vitrify) showed the highest viability.

Data courtesy of a customer

Conclusion

Cell Reservoir One (Vitrify) showed high viability with both 15 and 60 seconds of freezing protocol. With 60 seconds protocol, the survival rate of cells in Cell Reservoir One (Vitrify) was significantly higher than other freezing media.

	Freezing Medium	The Number of Colony		
		Vitrification Method		Slow Freezing Method
		60 Seconds	15 Seconds	
A	Cell Reservoir One (Vitrify)	672	563	-
B	DAP213	37	479	-
C	Company A	-	-	172

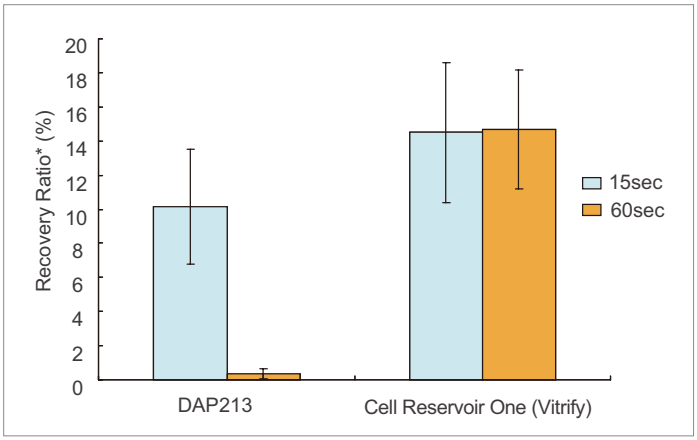
Application 2

Comparison of recovery ratio in primate stem cells

Cell Reservoir One (Vitrify) showed higher recovery ratio of Human iPS cells and Common Marmoset ES cells compared to DAP213.

- **Recovery ratio of Human iPS cells (253G1 cell line*) after vitrification**

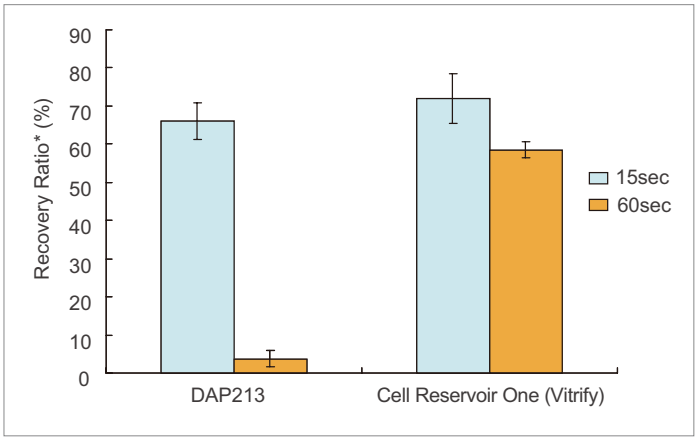
*Nakagawa, M. *et al. Nat Biotechnol*, 2008 Jan;**26**(1):101-106



Data courtesy of Fukui University

- **Recovery ratio of common marmoset ES cells (CMES 40 cell line) after vitrification**

*Sasaki, E. et al. *Stem Cells*, 2005 Oct; **23**(9):1304-1313



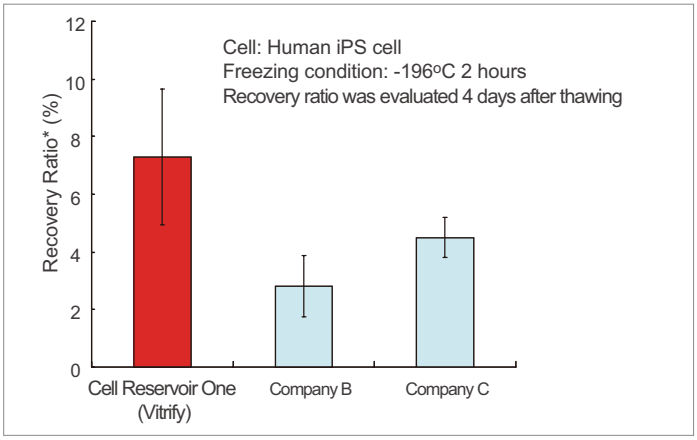
*Recovery ratio (%) =

$$100 \times \frac{\text{the number of colonies after vitrification and thawing}}{\text{the number of colonies at general passage}}$$

Application 3

Comparison of recovery ratio of Human iPS cells (253G1 cell line) with competitors'

Human iPS cells were cryopreserved for 2 hours with 15 seconds freezing protocol in each company's freezing medium. Recovery ratio was evaluated 4 days after thawing. Cell Reservoir One (Vitrify) showed the highest recovery ratio.



*Recovery ratio (%) =

$$100 \times \frac{\text{the number of colonies after vitrification and thawing}}{\text{the number of colonies at general passage}}$$

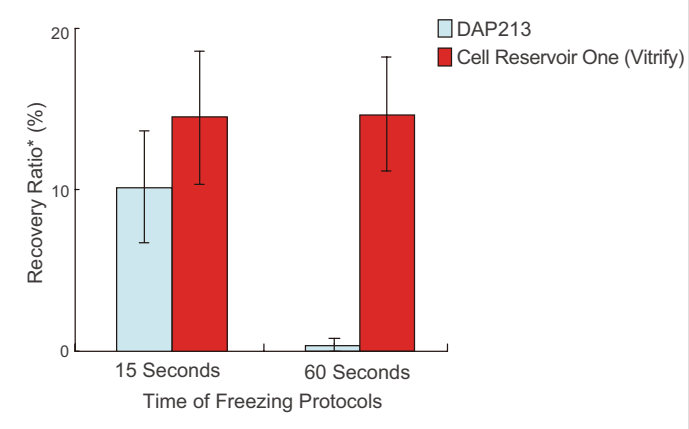
Data courtesy of Fukui University

Application 4

Comparison of recovery ratio in Human iPS cells (253G1 cell line) and examination of their undifferentiated state

Cell Reservoir One (Vitrify) showed higher recovery ratio compared to DAP213. The time of freezing protocols (15 seconds or 60 seconds) did not affect the recovery rate of cells in Cell Reservoir One (Vitrify). Expression of undifferentiated marker gene was also confirmed.

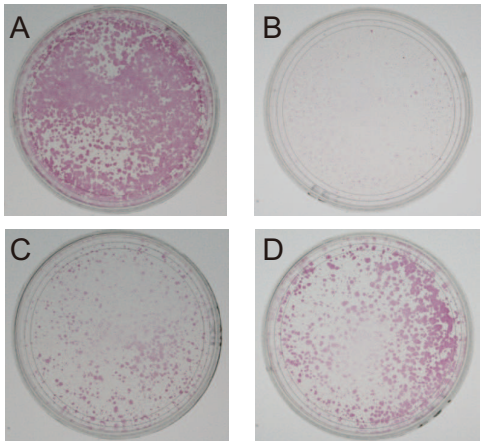
- **Recovery ratio**



*Recovery ratio (%) =

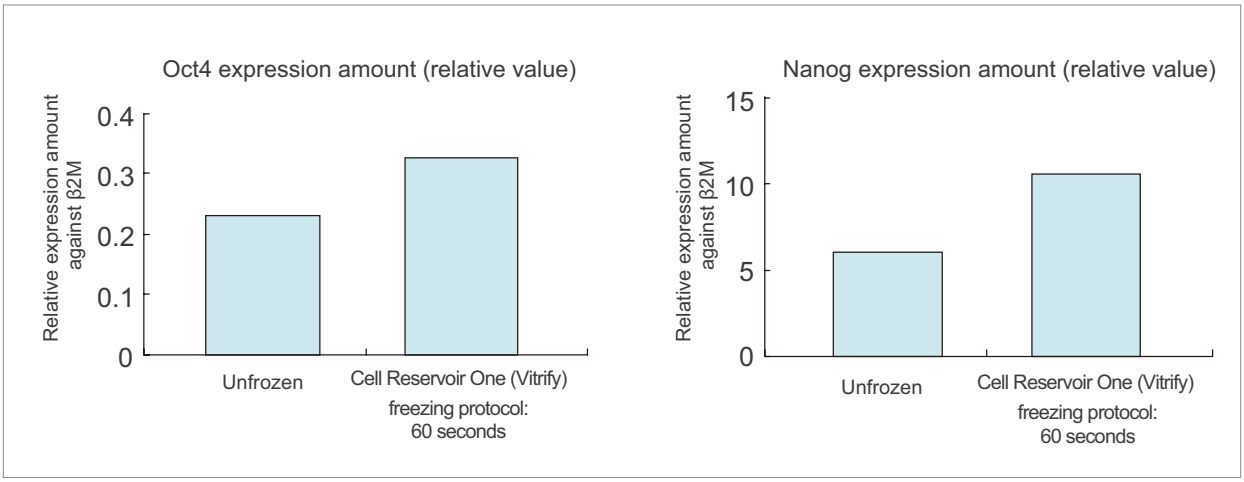
$$100 \times \frac{\text{the number of colonies after vitrification and thawing}}{\text{the number of colonies at general passage}}$$

- **Alkaline phosphatase activity**



A：Unfrozen
B：FBS/10%DMSO (Frozen by slow freezing method)
C：DAP213 (Vitrification method, 15 seconds freezing protocol)
D：Cell Reservoir One (Vitrify)
(Vitrification method, 15 seconds freezing protocol)

- **Expression of undifferentiated marker gene**



β2M: β2 microglobulin

Data courtesy of Fukui University

Protocol

Freezing protocol

1. Prepare tweezers and liquid nitrogen near a clean bench.
2. Detach primate ES/iPS cells with dissociation solution (0.25% trypsin/collagenase IV solution), and carefully collect the solution not to break down the cell colonies.
3. Aliquot the cell colonies into centrifuge tubes, and centrifuge them to remove as much supernatant as possible.
   *If Cell Reservoir One (Vitrify) is diluted by the remaining supernatant, the viability may decrease.
4. Add 200ul of Cell Reservoir One (Vitrify), and carefully mix by pipetting 4-5 times not to break down colonies. Transfer them quickly into a cryopreservation tube, and tighten a cap.
5. Immerse 2/3 height of the tube into liquid nitrogen for 10 seconds using tweezers, and then immerse it completely. For the successful cryopreservation, (4)-(5) process should be performed within 60 seconds.
6. Transfer the tube into a liquid nitrogen storage tank.

Thawing protocol

1. Pre-warm 10 ml of cell culture medium in a centrifugation tube at 37°C.
2. Take out the cryopreservation tube containing frozen cells from the liquid nitrogen storage tank, and transfer it to a clean bench leaving it immersed in liquid nitrogen.
3. Take out the tube from liquid nitrogen. Open a cap and discard liquid nitrogen in the tube by turning it upside down.
4. Thaw the cell quickly by adding more than 800ul of the pre-warmed cell culture medium to the tube and pipetting a few times.
   *The larger volume of medium is used, the quicker the sample thaws. Add suitable volume of medium depending on the tube size.
5. Transfer the cell suspension (4) to the centrifugation tube (1).
   *Operate (3)-(5) as quickly as possible.
6. Wash the cryopreservation tube with cell culture medium, and transfer the medium to the centrifugation tube (5).
7. Remove as much supernatant as possible after centrifugation, and seed cells in fresh medium.

Caution

1) Pretest the target cell before use.
2) NACALAI TESQUE, INC. takes no responsibility for any damages or losses arising from use of this product.

Ordering Information

Product Name	Grade	Storage	Product No.	PKG Size
Cell Reservoir One, Vitrify	SP	R	11325-62	25 ml

(Storage) R: Refrigerator

SERICIN, a major constituent of Cell Reservoir One, is produced by SEIREN.

Related Products

Product Name	Manufacturer	Storage	Product No.	PKG Size
Mitomycin C Solution (1mg/ml)	Nacalai Tesque	F	20898-21	1 ml
Y-27632		F	08945-71	1 mg
			08945-84	5 mg
FGF basic, Human Recombinant, animal free	Nacalai USA	F	11393-14	10 µg
			11393-56	50 µg
LIF, Mouse Recombinant		R	07695-81	1.0 ml (10^6 units/ml)
			07689-71	1.0 ml (10^7 units/ml)
LIF, Human Recombinant		R	07690-31	1.0 ml (10^6 units/ml)
			07692-11	1.0 ml (0.5×10^7 units/ml)
Cell Reservoir One Trial Set (w/ DMSO & w/o DMSO)	Nacalai Tesque	R	09550-01	1 set (10 ml each)
Cell Reservoir One (with DMSO)		R	07485-44	100 ml
Cell Reservoir One (without DMSO)		R	07579-24	100 ml

(Storage) R: Refrigerator F: Freezer

For research use only, not intended for diagnostic or drug use.

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