

# Experiment of scaling up in freezing and thawing of human iPS cells

With the development of cell processing products and regenerative medicine products, scaling up of cell culture processes has been required. However, there are concerns about the disadvantages of scaling up, such as decreased efficiency of culture work. WAKEN B TECH and Miltenyi Biotec collaborating with the research group of Dr. Shugo Tohyama, Department of Cardiovascular Medicine, Keio University School of Medicine conducted a study with 250 mL cryobag as a scaling up to reach the actual cell processing product storage volume, after the success with preliminary experiment using 50 mL cryobag in cell freeze-thaw and confirming cell growth. ThawSTAR CB, a cryobag thawing device and CryoMACS Freezing Bag were used for the study.



ThawSTAR CB

## Comparison experiment

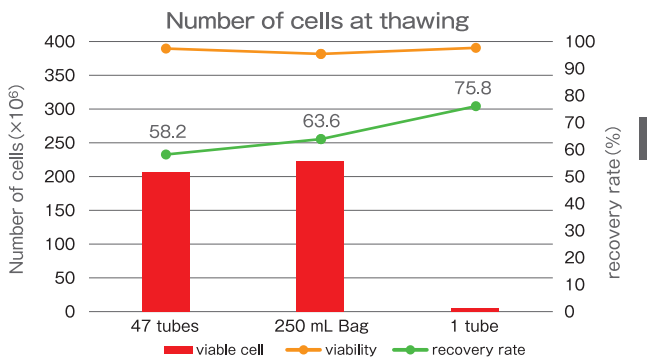
### 47 × 1.5 mL/tube vs max volume of 250 mL cryobag ( 70 mL )

[Cells] Human iPS cells  
 [Volume] 70 mL Cell Solution  
 [Freezing period] approx. 1.5 months  
 [Freezing medium] STEM-CELLBANKER®  
 [Concentration]  $5 \times 10^6$  cells/mL

	47×2 mL tubes*	250 mL bag	Control (1 tube)
labeling	20 min.	1 min.	1 min.
freezing	20 min.	7 min.	1 min.
thawing	90 min.	30 min.	13 min.
<b>Total</b>	<b>130 min.</b>	<b>38 min.</b>	<b>15 min.</b>

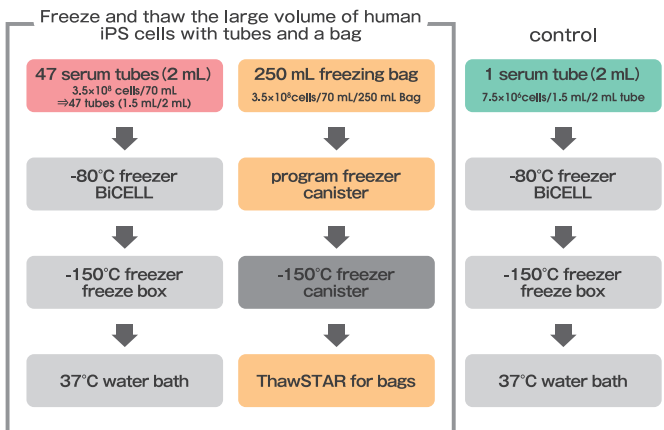
\* Assuming that 47 tubes of 2 mL serum tubes were operated, 5 tubes were frozen and thawed. Work time was calculated from the actual work time of  $5 \times 2$  mL serum tubes and assuming 2 people would work.

As the number of processed vials increases, the risk of contamination may increase.

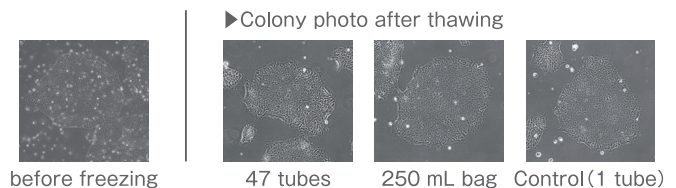
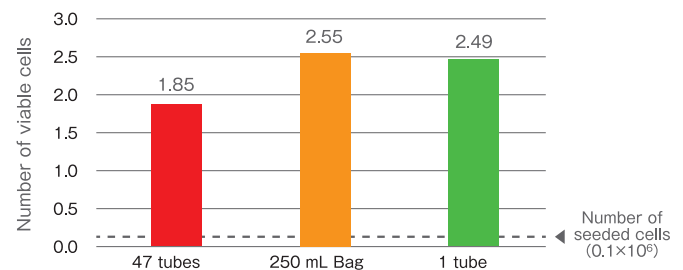


	47 tubes	250 mL bag	Control (1 tube)
Number of frozen cells (x10 <sup>6</sup> )	352	350	7.5
Number of viable cells (x10 <sup>6</sup> )	205.32	222.60	5.69
viability (%)	97.5	95.5	97.5
<b>*Recovery rate</b>	<b>58.2</b>	<b>63.6</b>	<b>75.8</b>

\* Recovery rate = viable cells after thawing/number of frozen cells × 100



$0.1 \times 10^6$  cells of the thawed cells were seeded on a 10 cm dish and cultured.



Result

Increased bag size from 50 mL to 250 mL for freezing and thawing of human iPS cells **succeeded in scaling up!!**

Damage from freeze-thaw **by using bag** **Reduced damage to human iPS cells!!**

Recovery rate, proliferation after thawing and work efficiency **compared to vials** **Better with bags!!**

Work time **by using bag** **Significantly shortened!!**

# Equipment available at WAKEN B TECH, can be adopted to manufacture and manage pharmaceutical products

## Cell freezing process



### ▶Cell freezing bag

CryoMACS freezing bag ( Miltenyi Biotec )

A safe and secure freezing bag that has been certified as a **medical device**.

There are sizes from 50 mL to 1000 mL, and it is a global standard bag that can be available all over the world.

Product Info



### ▶Protective bag sealer

Validatable Vacuum Sealer VMS163B ( SEAL Eito )

**Validatable** vacuum pack sealer. Seals the protective bag of the CryoMACS freezing bag without any problem and implements double packaging of samples.



### ▶Aluminum canister ( Fingallink, Custom Biogenic Systems )

In addition to the Fingallink product developed exclusively for CryoMACS, a versatile aluminum canister (manufactured by CBS) is also available.



### ▶Programmable freezer FZ-3100 ( Strex )

**IQOQ** applicable, programmable freezer using Stirling engine and no liquid nitrogen. This programmable freezer is optimal for airtight manufacturing sites such as CPC.

## Cell preservation and transportation process



### ▶Transport container

Ice bucket 4L, 9L ( Biocision )

Bucket with lid for high insulation and cleanliness. Compatible with dry ice and liquid nitrogen.

Product Info



### ▶Liquid nitrogen storage container Fusion ( MVE Biological Solutions )

**IQOQ** applicable

No need to replenish liquid nitrogen after the first fill.

It is a storage container with excellent running cost performance.

Product Info



## Cell thawing process



### ▶Frozen bag thawing device ThawSTAR CB ( BioLife Solutions )

**IQOQ** applicable device for optimal thawing without using water. A single unit can be used to thaw frozen bags from 50 mL to 750 mL.

Thawing time is within 10 minutes maximum.

Product Info



**These products can also be introduced for optimization at the bedside.**

For each product, please contact us.

\* All products in this catalog are for research use except CryoMACS freezing bags. Please note that they cannot be used for medical purposes.

\* Prices, appearance, accessories, etc. are subject to change without notice for improvement. Prices do not include consumption tax.

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