Automated and Water-free Thawing of Stem Cells using the ThermoMixer® C with the SmartBlock™ cryo thaw

Aurelie Tacheny[1], Silvia Tejerina Vargas[1], Blandine Vanbellinghen[1], Ines Hartmann[2]

Eppendorf Application Technologies[1], Eppendorf SE[2]

Corresponding author*: hartmann.i@eppendorf.de

Abstract

Thawing of stem cells by submerging the cryovial in a water bath is still the most common technique, although the procedure mostly lacks standardization and the risk of introducing a contamination is well known.

The Eppendorf ThermoMixer C in combination with the SmartBlock cryo thaw includes a program for automated thawing of cells, which allows highly convenient and reproducible thawing procedures.

By the absence of water, the risk of introducing a contamination during the process is reduced to a minimum. Here we show, that the ThermoMixer C is not only well suited for cell lines, but also for sensitive stem cells.

Introduction

It has previously been shown that the Eppendorf ThermoMixer C in combination with the Smart Block cryo thaw is well suited to thaw cell lines with the same high retrieval rate as the water bath [1].
It has also been shown that the thawing procedure is highly reproducible [1].
The small device footprint allows the use at the point of further processing, e.g. in or close to the biosafety cabinet and the SmartBlock cryo thaw can process multiple vials in parallel. That saves time and optimizes workflows.

Here we show that the automated thawing program is also well suited for stem cells, that are more sensitive to cryopreservation than cell lines [2].
We compare the standard water-bath thawing with the automated thawing method of using human induced pluripotent stem cells (hiPSCs) and human bone marrow derived mesenchymal stem cells (hMSC-BM). Cell morphology, viability and the maintenance of pluripotency or multi-lineage differentiation potential was analyzed.

Material and Methods

hiPSCs (A18944, Thermo Fisher Scientifc and AISC-0011, Coriell Institute) and hMSC-BM (PT 2501, Lonza) were pre-expanded and frozen in liquid nitrogen according to standard scientific procedures given for the specific cell type using 2 mL cryovials in a volume of 1 mL (Eppendorf SafeCode Vial or Corning).

To compare cell thawing performance, cells where thawed in parallel with the ThermoMixer C with the Smart Block cryo thaw program “thawing cells” and classic water bath immersion 2-3 min. Thawing at room temperature for 3 h served as negative control (data not shown).

Results

hiPSCs expansion and analysis of pluripotency:

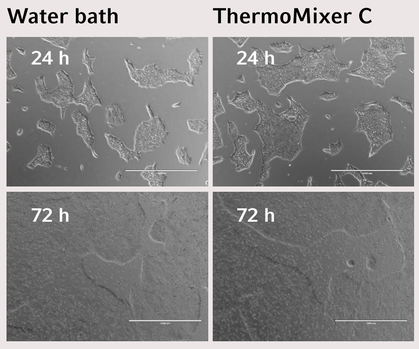
hiPSCs were cultivated on Matrigel-coated surface in a feeder-free adapted culture medium (Essential 8™ Flex Medium kit, A2858501, Thermo Fisher Scientific) according to supplier’s instructions and checked for cell morphology, spontaneous differentiation and cell growth 24 and 72 hours post-thawing.
Immunostaining was performed to confirm the maintenance of pluripotency after four successive passages for one hiPSC line (AISC-0011).

Cell counting was performed with the Vi-Cell cell counting device (Analis). Immunostainings was performed using the Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (A24881, Thermo Fisher Scientific). Fluorescent-stained cells were observed with the EVOS™ FL Cell Imaging System (Thermo Fisher Scientific).

1 hiPSCs show their typical morphology, viability and maintain their pluripotency

Cell Morphology

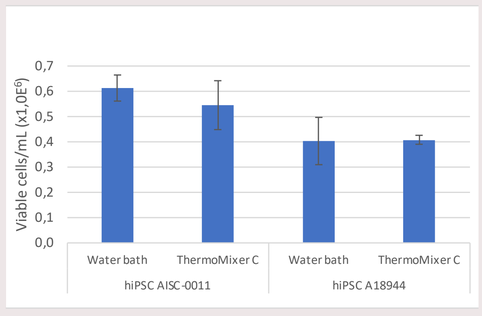
(A)



hiPSCs 24 and 72 hours post-thawing show their typical and expected morphology and confluences with both thawing methods. No spontaneous differentiation or spontaneous detachment was observed. Depicted is the hiPSC (AISC-0011) (Magnification 100x). The hiPSC line (A18944) showed similar results. Thawing at room temperature resulted in smaller clumps and lower confluence (data not shown)

Viable Cell Count

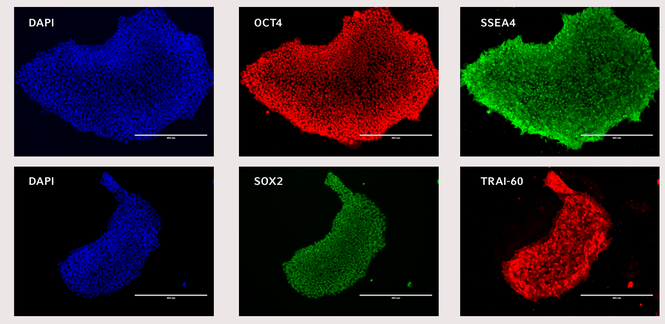
(B)



hiPSCs viable cell counts 72 hours post-thawing confirmed the microscopic observation. The cells thawed with the ThermoMixer C showed the same proliferation as the cells thawed with the water bath. Cells thawed at room temperature showed a much lower number of viable cells (data not shown)

Analysis of Pluripotency Marker Expression

(C)



hiPSCs immunostaining results of hiPSC (AISC-0011) thawed with the ThermoMixer C confirm the maintenance of pluripotency after 4 successive passages post-thawing (Magnification 100x)
(Similar results with the water bath, data not shown)

hMSC-BM expansion and multi-lineage differentiation:

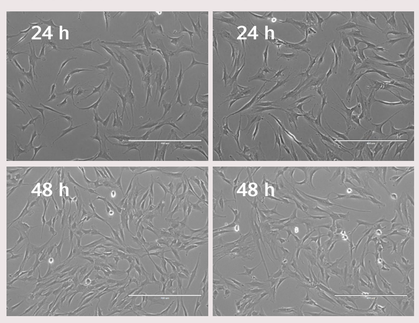
hMSCs were cultivated in a serum-free adapted culture medium (Mesenchymal Stem Cell Growth Medium Bulletkit, PT-3001, Lonza) according to supplier’s instructions and checked for cell morphology and viability 24- and 48-hours post-thawing.

Osteogenic and adipogenic differentiations have been induced after one successive passage by using the OsteoMax-XFTM Kit (Human) (SCM121, Merck-Millipore) and the hMSC Adipogenic Differentiation Bulletkit™ (PT-3004, Lonza). Non-induced cells have been used as a negative control for differentiation. Respective differentiation efficiencies have been assessed through Alizarin Red and Oil Red O.

2 hMSCs show their typical morphology, viability and their multi-lineage differentiation potential

Cell Morphology

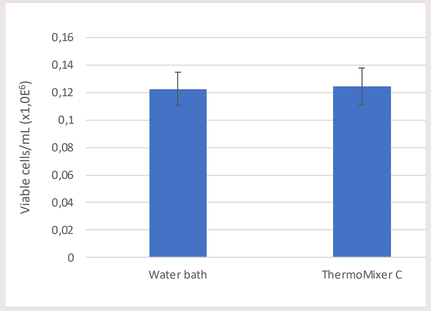
(A)



hMSCs 24 and 48 hours post-thawing show their typical and expected morphology and confluences with both methods. No spontaneous differentiation or spontaneous detachment was observed. (Magnification 100x). Thawing at room temperature resulted in lower confluence (data not shown)

Viable Cell Count

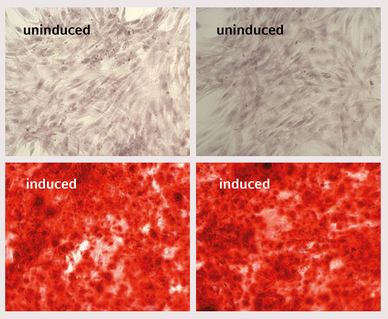
(B)



hMSCs 48 hours post-thawing confinned the microscopic observation. The cells thawed with the ThermoMixer C showed the same proliferation as the cells thawed with the water bath. Cells thawed at room temperature showed a much lower number of viable cells (data not shown). Results represent a mean of two vials (n-2)

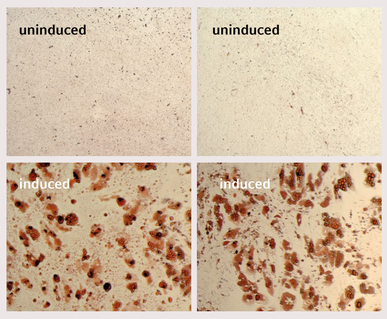
Differentiation Ability

(C) Oestogenic



Alizarin Red staining 15 days post-osteogenic induction confirm a high osteogenic differentiation level in contrast to non-induced cells, as suggested by the intense Alizarin red coloration (Magnification 200x)

(C) Adipogenic



Oil red O staining 17 days postadipogenic induction confirm a high differentiation level in contrast with non-induced cells, as suggested by the accumulation of intracellular lipid droplets colored by Oil red O staining (Magnification 200x)

Summary

> hiPSCs and hMSCs thawed with the ThermoMixer C showed similar fast recoveries, cell viabilities and growth patterns compared to the classic water bath method. In addtion, it was shown, that pluripotency or multipotency was maintained.

> The ThermoMixer C allows water-free, standardized thawing and parallel handling of multiple vials. With the small footprint, thawing can be done at the place of further processing. The SmartBlock system allows flexible workflow integration.

References

1. Tacheny A, Tejerina Vargas S, Chandelier N, Hoet JF, Karow K, Hartmann I. Standardized and Water-free Cell Thawing using the Eppendorf ThermoMixer® C with the Eppendorf SmartBlock™ cryo thaw, APPLICATION NOTE I No. 437, www.eppendorf.com
2. Thompson M, Kunkel E, Ehrhard. Standardized Cryopreservation of Stem Cells. In Stem Cell Technologies in Neuroscience. Neuromethods 10.10 07/ 978-1- 4939-7024-7_13. 2017

Your local distributor: www.eppendorf.com/contact · Eppendorf SE · 22331 Hamburg · Germany · eppendorf@eppendorf.com · www.eppendorf.com