APPLICATION NOTE | No. 452

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

Aurélie Tacheny[1], Silvia Tejerina Vargas[1], Jean-François Hoet[1], Blandine Vanbellinghen[1], Ines Hartmann[2]

[1]Eppendorf Application Technologies, S.A., Namur, Belgium
[2]Eppendorf SE, Hamburg, Germany

Abstract

Thawing of stem cells by submerging the cryovial in a water bath is still the most common technique, although the procedure often 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. The absence of water reduces the risk of introducing contaminations during the thawing process. Here we show, that the Eppendorf ThermoMixer C is not only well suited for thawing of cell lines, but also for sensitive stem cells.

Introduction

The Eppendorf ThermoMixer C in combination with the SmartBlock cryo thaw is well suited for thawing of cell lines with the same high retrieval rate as a water bath [1]. The thawing procedure is also highly reproducible [1]. The SmartBlock cryo thaw can process up to 24 vials in parallel and the small footprint of the ThermoMixer C (20.6 x 30.4 cm; 8.1 x 12.0 in) allows using the device at the point of further sample processing, e.g. in or close to a biosafety cabinet. That saves time and optimizes workflows.

In this application note we show that the SmartBlock cryo thaw is also well suited for thawing of stem cells, which are more sensitive to cryopreservation than cell lines [2]. We compare the results of thawing human induced pluripotent stem cells (hiPSCs) and human bone marrow derived mesenchymal stem cells (hMSC BM), either in a conventional water bath or in a ThermoMixer C equipped with a SmartBlock cryo thaw. Cell morphology, viability and the maintenance of respective differentiation efficiencies were analyzed.

The Eppendorf ThermoMixer C in combination with the SmartBlock cryo thaw features an integrated program for automated thawing of mammalian cells. For easy and standardized cell thawing procedures with minimized contamination-risk. Optimized for 1.8 – 2 mL cryovials and 1 mL fill volume.

Materials and Methods

hiPSCs (A18944, Thermo Fisher Scientific; AISC-0011, Coriell Institute) and hMSC-BM (PT 2501, Lonza®) were pre-expanded and frozen in liquid nitrogen using 2 mL cryovials (Eppendorf SafeCode Vial or Corning®) in a volume of 1 mL cryomedia. hiPSCs were frozen in mFreSR™ cryopreservation solution (Stem Cell Technologies, 05854) and hMSCs-BM in Mesenchymal Stem Cell Basal Medium (Lonza # PT3238) supplemented with 20 % FBS and 10 % DMSO. To compare cell thawing performance, cells where thawed with the Eppendorf ThermoMixer C with the SmartBlock cryo thaw program for thawing cells (set thaw time of 3 minutes) and in parallel by classic water bath immersion. Tests were run in triplicates. Cell specific performance sheets are available on www.eppendorf.com/ThermoMixer.

hiPSCs expansion and analysis of pluripotency
hiPSCs were recovered in medium drop-wise and cryoprotectant was removed by centrifugation at 200 x g for 5 min at room temperature. The cell pellet was resuspended in a feeder-free adapted culture medium (Essential 8™ Flex Medium kit, A2858501, Thermo Fisher Scientific) supplemented with RevitaCell® (Thermo Fisher Scientific, A2644501) according to suppliers' instructions and seeded on a Matrigel®-coated surface (Corning Inc.). Cell morphology, the absence of spontaneous differentiation and cell growth was analyzed at short-term (24 and 72 hours post-thawing) and long-term (until passage 4). From 24h post-seeding, a microscopic inspection and a medium refreshment was performed each 24 or 48 hours. About 3-4 days were required between two successive passages. Cell counting was performed with the Vi-Cell™ cell counting device (Analis). Immunostaining was performed to confirm the maintenance of pluripotency after four successive passages for one hiPSC line (AISC-0011) using the Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (A24881, Thermo Fisher Scientific) according to manufacturer's recommendations. Fluorescent-stained cells were observed with the EVOS™ FL Cell Imaging System (Thermo Fisher Scientific).

hMSCs-BM expansion and multi-lineage differentiation
hMSCs-BM were recovered in medium drop-wise and cryoprotectant was removed by centrifugation at 200 x g for 5 min at room temperature. Cells were seeded on TCT-surface in a serum-free containing adapted culture medium (Mesenchymal Stem Cell Growth Medium Bulletkit™, PT-3001, Lonza) according to supplier's instructions, incubated at 37°C and 5 % CO_2 and analyzed for cell morphology and viability 24- and 48-hours after thawing. Osteogenic and adipogenic differentiations have been induced after one successive passage by using the OsteoMax-XF™ Kit (Human) (SCM121, Merck-Millipore®) and the hMSC Adipogenic Differentiation Bulletkit™ (PT-3004, Lonza) according to supplier's recommendations. Non-induced cells have been used as a negative control for differentiation. Respective differentiation efficiencies have been assessed through specific staining's. The osteogenic differentiation and mineralized matrix accumulation have been highlighted by Alizarin Red staining performed 15 days post-induction. The intracellular lipid droplet accumulation associated to the adipogenic differentiation has been confirmed by Oil Red O staining performed 17 days post-induction.

Results and Discussion

hiPSCs expansion and maintenance of pluripotency
The hiPSCs thawed with the water bath or the Eppendorf ThermoMixer C showed the typical and expected hiPSC morphology 24 and 72 hours post-thawing forming flat, tightly packed colonies with well-defined boarders. (Figure 1). No abnormalities in shape or densities were visible. 72 hours post-thawing, cells thawed with the Eppendorf ThermoMixer C formed a confluent monolayer as well as the cells thawed with the water bath.

The viable cell count performed 72 hours post–thawing confirmed the microscopic observation. The hiPSCs thawed with the Eppendorf ThermoMixer C showed the same proliferation as the cells thawed with the water bath (Figure 2).

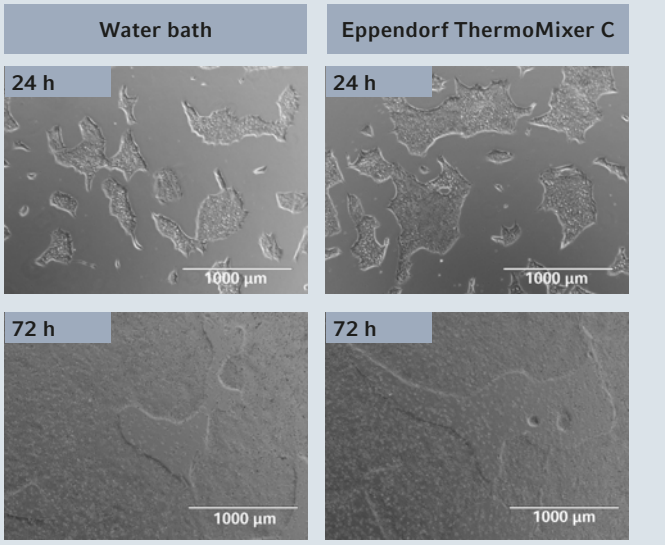

Figure 1: hiPSCs 24 and 72 hours post-thawing show their typical and expected morphology and confluences with both thawing methods. No spontaneous differentiation or spontaneous detachment was observed. Depicted is the hiPSC (AISC-0011). The hiPSC line (A18944) showed similar results.

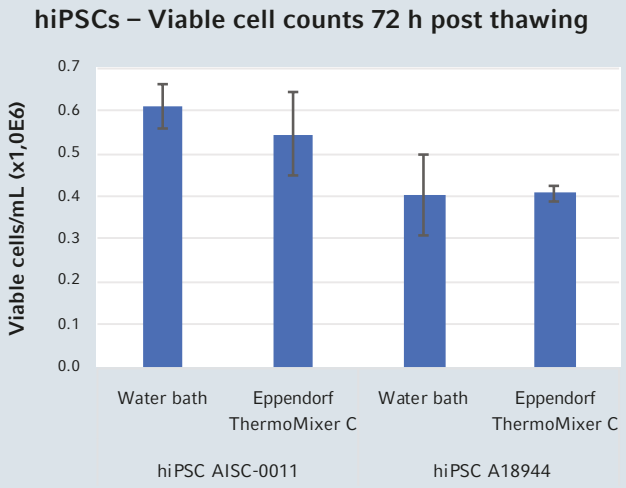

Figure 2: Viable cell counts of hiPSCs 72 hours post-thawing. Results represent a mean of two counted vials (n=2).

Cells were passaged until passage four. A normal proliferation rate and morphology was observed during all passages (data not shown). No spontaneous differentiation or spontaneous detachment was observed during all conditions.

hiPSCs have the ability to differentiate into any type of cell lineage. To confirm this maintenance of pluripotency, immunostaining was performed after four successive passages for the hiPSC line (AISC-0011) in addition. Immunostaining results confirm the maintenance of pluripotency for the cells thawed with the Eppendorf ThermoMixer C (Figure 3) and the water bath (data not shown).

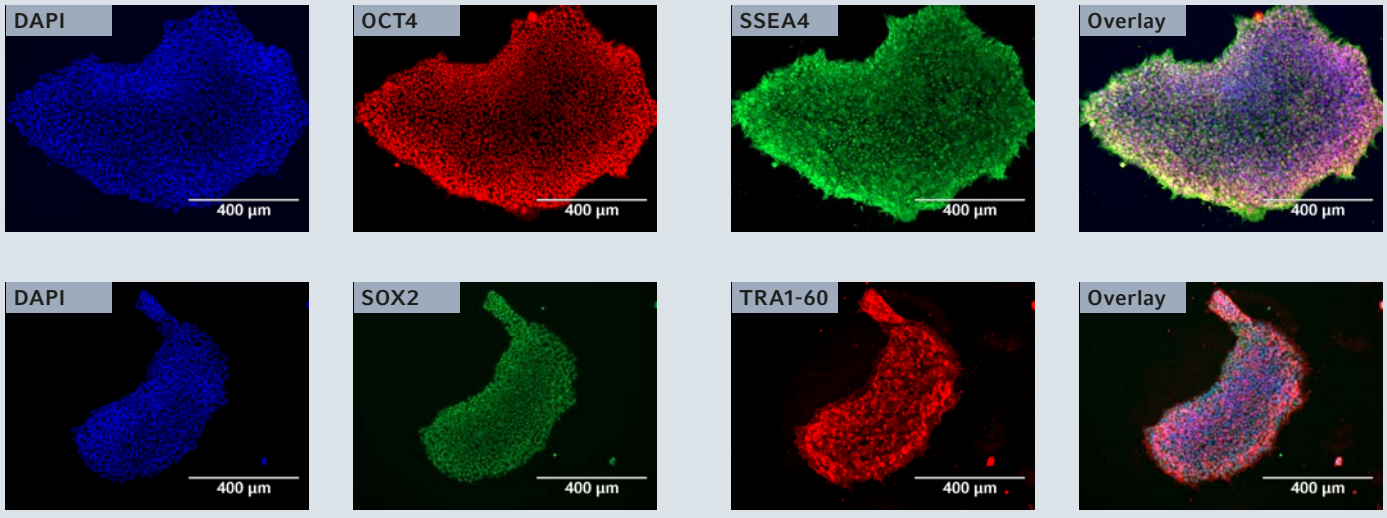

Figure 3: hiPSCs Immunostaining results of hiPSC thawed with the Eppendorf ThermoMixer C confirm the maintenance of pluripotency after 4 successive passages post-thawing (Similar results were obtained with the water bath, data not shown)

hMSC-BM expansion and differentiation potential

The hMSCs-BM showed their typical and expected morphology after thawing with both methods as depicted in figure 4. No abnormalities in shape or densities were visible. No spontaneous differentiation or spontaneous detachment was observed during all conditions.

The viable cell count performed 48 hours post–thawing confirmed the microscopic observation (Figure 5). The cells thawed with the Eppendorf ThermoMixer C showed the same proliferation levels as the cells thawed with the water bath.

hMSCs-BM belong to the category of adult stem cells with the ability to differentiate into specialized cell types present in a specific tissue or organ. To confirm this multilineage differentiation ability, two lineages, the osteogenic and adipogenic pathways, have been induced and differentiation ability was confirmed by immunostaining (Figure 6 and 7), respectively. At the qualitative level, the differentiation potential is comparable between cells thawed with the Eppendorf ThermoMixer C and the water bath.

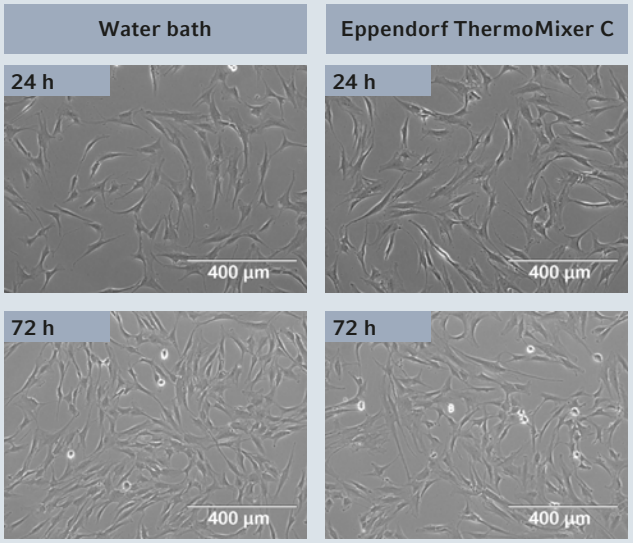

Figure 4: hMSCs-BM 24 and 48 hours post-thawing show the typical and expected morphology and confluences with both thawing methods.

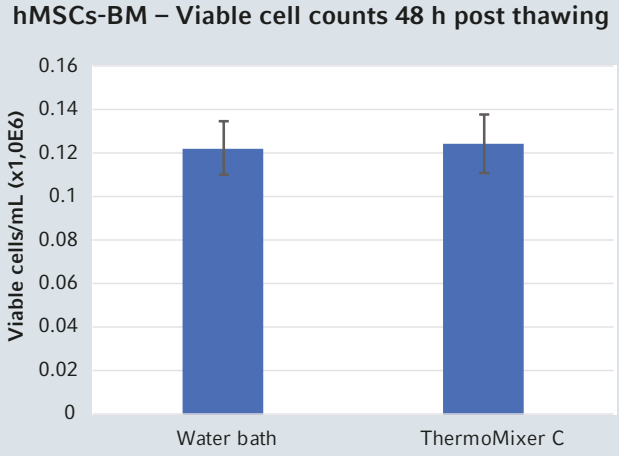

Figure 5: Viable cell counts of hMSCs-BM 48 hours after thawing. Results represent a mean of two vials (n=2).

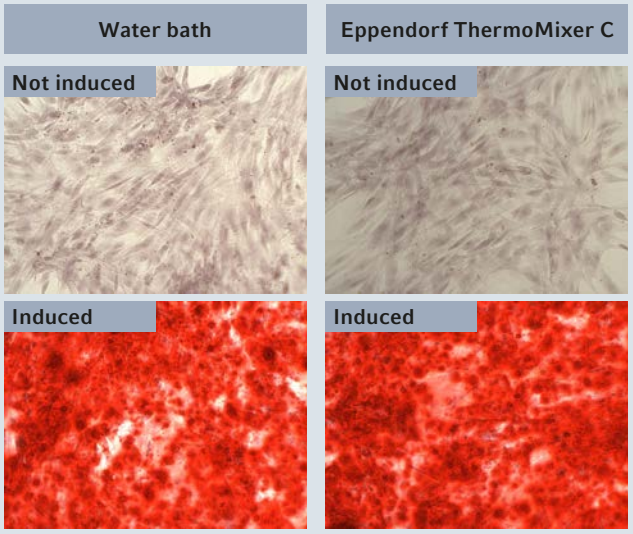

Figure 6: Alizarin Red staining of hMSCs-BM 15 days post-osteogenic induction. Cells thawed with both methods present a high differentiation level in contrast with non-induced cells, as suggested by the intense Alizarin red coloration (magnification 200x).

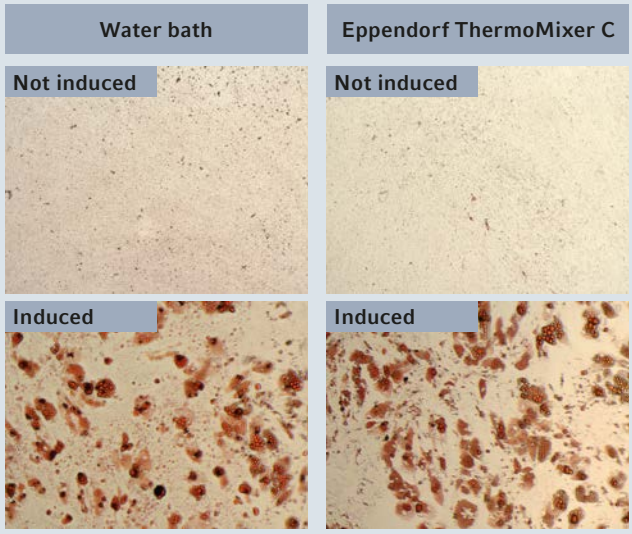

Figure 7: Oil Red O staining staining of hMSCs-BM 17 days post-Adipogenic induction. Cells thawed with both methods present a high differentiation level in contrast with non-induced cells, as suggested by the accumulation of intracellular lipid droplets colored by Oil Red O staining (magnification 200x).

In summary, both stem cell types showed similar fast recovery rates, cell viability and growth patterns when thawed with the Eppendorf ThermoMixer and a water bath. In addition, we could show, that pluripotency for hiPSCs and multipotency for hMSCs were at the same level with both thawing methods. The cell tests clearly show that the automated thawing with the Eppendorf ThermoMixer C in combination with the SmartBlock cryo thaw is well suited not only for cell lines but also for sensitive stem cells.

Conclusion

The results show that the Eppendorf ThermoMixer C with the SmartBlock cryo thaw is well suited to thaw even sensitive stem cells with the same high retrieval rate and preservation of differentiation potential as a water bath. It offers highly reproducible thawing procedures and can handle multiple vials in parallel. The handling is easy with the pre-set program and the device can be integrated flexibly in workflows with its small device footprint and exchangeable SmartBlock system. The contamination risk that is associated with water bath immersion is eliminated, as the method is water-free. The ThermoMixer C with the SmartBlock cryo thaw offers an attractive improvement for general stem cell handling.

Literature

[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* 10.1007/978-1-4939-7024-7_13. 2017

Ordering information

Description	International Order no.	North American Order no.
Eppendorf ThermoMixer® C, basic device without Eppendorf SmartBlock, 220 V–240 V	5382 000.015	–
Eppendorf ThermoMixer® C, basic device without Eppendorf SmartBlock, 110 V–130 V	–	5382000023
Eppendorf SmartBlock cryo thaw, thermoblock for 24 cryo tubes, 2.0 mL, all base shapes	5318 000.001	5318000001
Eppendorf CryoStorage Vial 2.0 mL, pre-capped, pre-racked, 10x48 vials, 2D SafeCode, sterile	0030 079.485	0030079485

Your local distributor: www.eppendorf.com/contact
Eppendorf SE · Barkhausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com