Cell type: Episomal Human iPSC

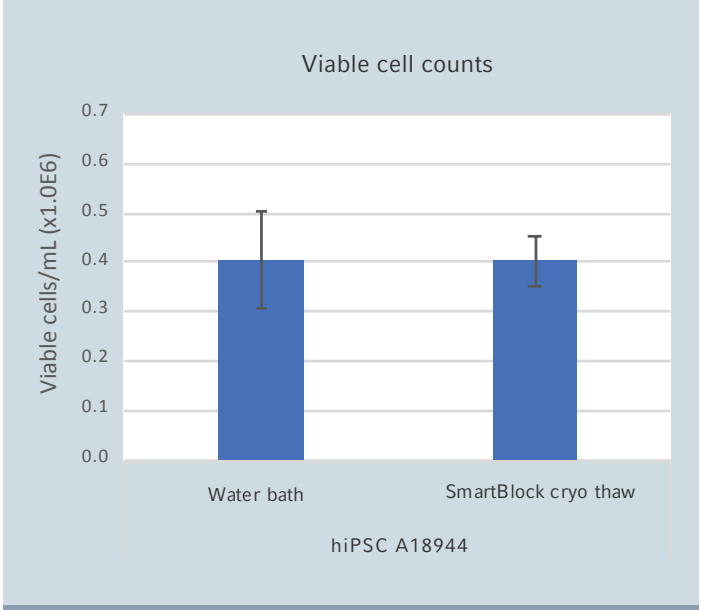
(Thermo Fisher Scientifc, A18944)

Performance of the SmartBlock™ cryo thaw for cell thawing

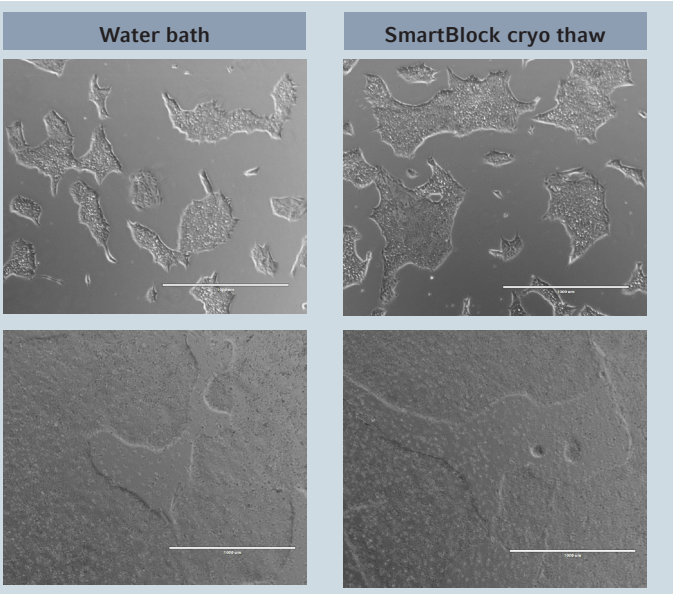
Freezing medium: mFreSR™ cryopreservation solution (Stem Cell Technologies, 05854)

Procedure of freezing: Cells were frozen when reaching a confluency of 60-70 % in 1 mL of cold (2-8 °C) mFreSR medium in Eppendorf CryoStorage Vials 2.0 mL (0030 079.485). Cells were frozen using a slow rate-controlled cooling freezing container (-1°C/minute) (Sigma Aldrich, CLS432000-1EA) at -80 °C for the first 24 hours and later transfer to a liquid nitrogen container in the vapor phase (-135 °C) for long term storage.

Procedure of thawing: The vials were thawed using the program 'Thawing cells' (3 minutes, 500 rpm). Parallel thawing with a water bath was done. After thawing, cells were suspended in 10 mL Essential 8™ Flex Medium (Thermo Fisher Scientific, A2858501) and centrifuged at 200x g for 5 min. The cell pellet was resuspended in medium supplemented with RevitaCell™ (Thermo Fisher Scientific, A2644501) and cultivated on Matrigel® (Corning®, 354277) in a 6-well cell culture plate in 2 ml supplemented medium. Cell aggregates were incubated at 37 °C and 5 % CO_2. Medium refreshment was performed each 24 or 48 hours. About 3-4 days were required between two successive passages.



Cell counting was done with the Vi-CELL™ cell counter (Analis). Bars represent average count of three wells.



Representative Phase-contrast microscope images 24 h and 72 h post-thawing (40× and 100× magnification)

The program 'Thawing cells' of the Eppendorf SmartBlock cryo thaw allows optimal thawing and homogeneous growth of Episomal Human iPSC cells compared to the existing method (here: water bath).

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