amsbio
Part of Europa Biosite

Cryopreservation of organoids

Cell Freezing Media

Reliable organoid preservation starts here

- *GMP-compliant*
- *Chemically defined*
- *Exceptional post-thaw performance*

Organoid cryopreservation

Benefits

- Useful for drug sensitivity assays
- Maintains genetic information
- Share libraries easily
- Expand tractable cell line availability
- CRISPR compatible

Human airway organoids cryopreserved in CELLBANKER® 1

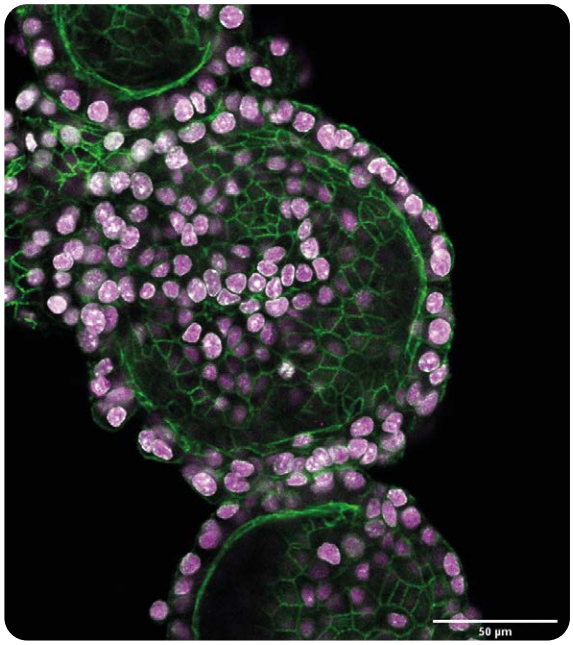

Fig. 1. Murine Thyroid Organoids frozen in CELLBANKER® 1 during the lockdown and imaged post-thaw. IF staining for DAPI, Pax8 (marker of thyroid gland organogenesis), and Phalloidin (white/magenta/green).

Image courtesy of the Clevers Lab, Hubrecht Institute.

Dr. Jelte van der Vaart, whilst a PhD student in the world famous Clevers lab*, utilised CELLBANKER® to simply and safely cryopreserve his organoids as we were entering the first COVID-19 lockdown.

"Indeed, before I was always using the FBS/DMSO based freezing method which not only costs a lot of time but also requires transfer by slow cooling to -80°C to liquid nitrogen.

On the day of the lockdown, me and some other researchers froze our organoids down for the first time in CELLBANKER®. The procedure was a lot easier and faster since I could simply transfer the organoids in CELLBANKER® to the freezer immediately.

This also solved the limited availability of Mr Frosty's which was a problem because we all had to freeze down lines.

After the lockdown, the organoids looked similar after thawing to standard freezing methods and could be easily retrieved from personal drawers instead of shared Mr Frosty drawers."

Jelte van der Vaart, PhD

** Dr. Jelte van der Vaart is currently a postdoctoral researcher, University Medical Centre Utrecht, Netherlands.*

"The presence of KRT5+ cells shows that the lung airway organoids are still consistent of stem cells which can be propagated into more organoids, in short the culture is still able to expand. This is an important message as well for the thawing that basal cells survive the freezing."

Jelte van der Vaart, PhD

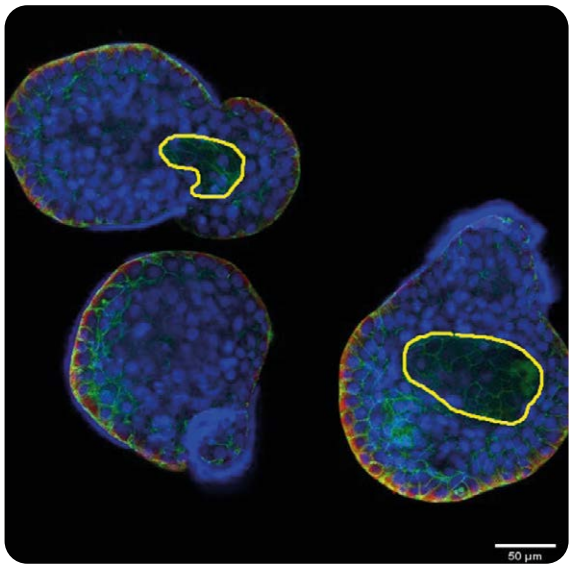

Fig. 2. Human Airway Organoids frozen in CELLBANKER® 1 during lockdown and imaged post-thaw. IF for DAPI, KRT5 (basal cell marker), Phalloidin (blue/red/green). Lumens highlighted in yellow.

Images courtesy of the Clevers Lab, Hubrecht Institute.

Article spotlight

Take a look at our **Thought Leadership Q&A** with Dr. Jelte van der Vaart discussing how to **simplify** and **standardise** your organoids cryopreservation, and the importance of **GMP-approved** cryopreservation for regenerative medicine applications!

A snippet from the article:

"The latest technology, like Amsbio's line of cryopreservation media, is ready-to-go and designed with regulatory conditions in mind, making it more convenient, reliable, and preferable for developing therapeutic applications that have stringent requirements for experimental control and documentation."

Organoid cryopreservation

Human bronchial organoids cryopreserved in STEM-CELLBANKER®

Uninfected

Infected with SARS-CoV-2

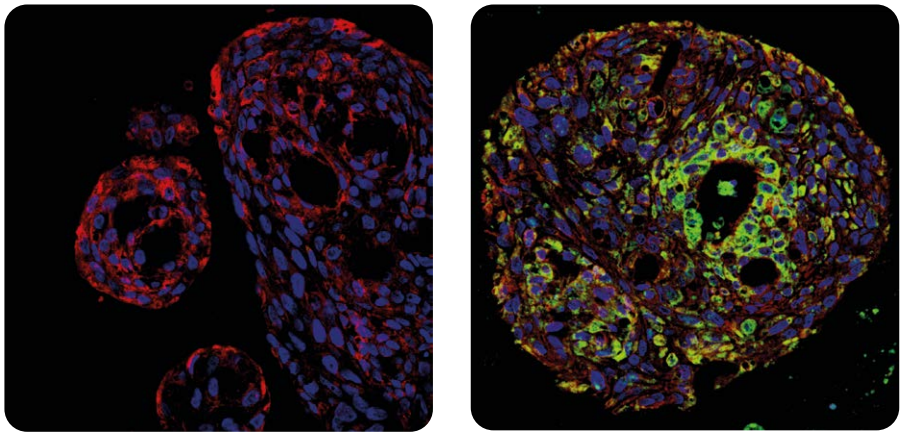

Fig. 3. Human bronchial organoids (uninfected and infected) after cryopreservation in STEM-CELLBANKER®. Red: acetylated α-tubulin, green: SARS-CoV-2-N protein, blue: DAPI.

Images courtesy of Kazuo Takayama (CiRA, Kyoto University, Japan).

Dr. Kazuo Takayama at Kyoto University, Japan, utilized human bronchial organoids to model SARS-CoV-2 infection, and ensured high viability and recovery upon thawing by using STEM-CELLBANKER® cryopreservation media.

"STEM-CELLBANKER® is an essential resource for freezing our bronchial organoids."

Dr. Kazuo Takayama, Kyoto University, Japan

** Dr. Kazuo Takayama is a Professor at the Department of Synthetic Human Body System, Medical Research Institute, Kyoto University, Japan.*

Human liver organoids stored in CELLBANKER® series

"Day 2 and day 4 images clearly show that organoids recovered and grew well in these three CELLBANKER® freezing media."

*Meritxell Huch, Gurdon Institute, Cambridge, UK**

** Meritxell Huch is currently a Group Leader at the MPI Molecular Cell Biology and Genetics, Dresden, Germany.*

	Day 2	Day 4	Organoid Growth Following Freezing
STEM-CELLBANKER® GMP grade			✔
CELLBANKER® 2			✔
CELLBANKER® 1			✔

CELLBANKER® cell freezing media

- *Consistently high cell viability post thaw (>90%)*
- *No programmed freezer or liquid nitrogen required*
- *Long shelf life*
- *Tested on many cell types*
- *Freeze directly in -80°C freezer*
- *Allows viable long term cell storage >10 years at -80°C or -196°C*
- *Ready-to-use formulas avoid mixing of DMSO and filtration*

CELLBANKER® 1

- Ready-to-use serum-containing cell cryopreservation medium.
- Suitable for a range of mammalian cell cultured, organoids, & tissues.
- High viability after freeze-thaw cycles, even for sensitive cells.

CELLBANKER® 2

- Ready-to-use serum-free cell cryopreservation medium.
- Optimised for serum-free cultured cells and peptide/protein expressing cells.
- No risk of contamination from serum.

Typical cell recovery rates for CELLBANKER® 1 and 2

Cell Type	Preservation Period (year)	Viability of cells (%) at -80°C
Fibroblasts	5	90
Caco-2	5	90
K562	5	90
Jurkat	10	90

Follow our CELLBANKER® protocol for the best results!

Read our protocol with best practice for freezing and thawing your organoids with CELLBANKER® to achieve optimal results here!

CELLBANKER® cell freezing media

Available in DMSO Free

STEM-CELLBANKER® GMP grade

- Chemically defined, animal-free, GMP-compliant stem cell cryopreservation solution, for basic research and in the clinical application of cell therapy products.
- Manufactured in compliance with JPN, EU, US and PIC/S GMP guidelines.
- Validated for cryopreservation of organoids and tissues with high viability.
- Optimised for stem cells and iPS cells storage.
- Available in DMSO-containing and DMSO-free formulations.

STEM-CELLBANKER® EX GMP-grade

- Chemically defined, xeno-free, GMP-compliant cryopreservation media.
- All components are approved for intravenous application, streamlining your clinical translation.
- Optimised for applications in regenerative medicine: no requirement for washing of the cryopreservation solution upon delivery of the cell therapy product.

Drug Master Files have been registered with the FDA for both STEM-CELLBANKER® and STEM-CELLBANKER® EX, for streamlined clinical translation!

Summary table of our different cryopreservation media formats:

Freezing solution	Serum-free	Animal-free	GMP-compliant	DMSO-free	Validated for use with organoids? (Lab PI name)
CELLBANKER® 1	✗	✗	✗	✗	Huch, Clevers
CELLBANKER® 2	✓	✓	✗	✗	Huch, Vallier, Forbes
STEM-CELLBANKER® GMP Grade	✓	✓	✓	✗	Huch, Klein, Takayama
STEM-CELLBANKER® DMSO-free, GMP Grade	✓	✓	✓	✓	Not yet
STEM-CELLBANKER® EX GMP-grade	✓	✓	✓	✗	Not yet

Featured citations

Article spotlight

Thought Leadership Piece in *Today's Clinical Lab* (Jun 29, 2022: 3 min read) **Simplifying and Standardizing Organoid Cryopreservation**. Written by Zahraa Chorghay, PhD.

CELLBANKER® 1

Clevers lab (Hubrecht Institute, Netherlands) - Long term cryopreservation of different types of organoids derived from cells of the airway and the thyroid to study associated diseases.

- van der Vaart, J., *et al.* (2021), *EMBO reports*, 22(12), e52058.
- Lamers, M. M. *et al.* (2021), *The EMBO journal*, 40(5), e105912.
- van der Vaart, J., *et al.* (2021), *Proceedings of the National Academy of Sciences*, 118(51), e2117017118.
  - (J.van der Vaart, personal communication).
- Van der Vaart, J., (2022) Modelling of a wide variety of diseases using adult stem-cell-derived organoids. (Doctoral dissertation, Utrecht University). PhD thesis available online for download as PDF from uu.nl.

Mayhew Lab (Cincinnati Children's Hospital, USA) - Cryopreservation of pellets of hPSC-derived mid-hindgut endoderm (MHE), subsequently aggregated into small intestinal organoids (HIOs).

- Pitstick, A. L., et al. (2022), *Stem Cell Reports*, 17(8), 1889-1902.

CELLBANKER® 2

Vallier lab (Cambridge, UK) - Cryopreservation of cholangiocyte organoids.

- Tysoe, O. C. *et al.* (2019), *Nat Protoc*, 14(6), 1884-1925.

Stuart Forbes (CRM, IRR, University of Edinburgh, UK) - Freezing primary liver cells from tissue samples, and the subsequent organoids cultured from these cells.

- Hallett, J. M. *et al.* (2022), *Cell stem cell*, 29(3), 355-371.e310.

Knoblich lab (Vienna, Austria) - Archiving clones with desired genetic characteristics.

- Bagley, J. A. *et al.* (2017), *Nature Methods*, 14(7), 743-751.
- Bajaj, S. *et al.* (2021), *The EMBO Journal*, 40(23), e108714.

STEM-CELLBANKER®

Takayama lab (CiRA, Japan) - Cryopreservation of SARS-CoV-2 model organoids.

- Sano, E. *et al.* (2022), *Communications Biology*, 5(1), 516.

Allon Klein lab (Harvard, USA) - Cryopreservation of Intestinal organoids.

- Tallapragada, N. P. *et al.* (2021), *Cell Stem Cell*, 28(9), 1516-1532.e1514.

Takenaka lab (Osaka International Cancer Institute, Japan) - Cryopreservation of Organoids from Human Epithelioid Sarcoma.

- Wakamatsu, T. *et al.* (2022), *Front Oncol*, 12, 893592.

THE CELLBANKER® RANGE

Description	Pack Size	Cat. Number
CELLBANKER® 1 - Serum containing	20 ml	11911
CELLBANKER® 1 - Serum containing	100 ml	11910
CELLBANKER® 2 - Serum free	20 ml	11915
CELLBANKER® 2 - Serum free	100 ml	11914
STEM-CELLBANKER® - GMP	20 ml	11922
STEM-CELLBANKER® - GMP	100 ml	11924
STEM-CELLBANKER® - GMP - DMSO - Free	20 ml	13925
STEM-CELLBANKER® - GMP - DMSO - Free	100 ml	13926
STEM-CELLBANKER® EX	100 ml	11936
CELLOTION® Cell Wash Solution	100 ml	11918

Amsbio is the global source for CELLBANKER® reagents. CELLBANKER® is a registered trade mark of and manufactured by NIPPON ZENYAKU KOGYO.

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