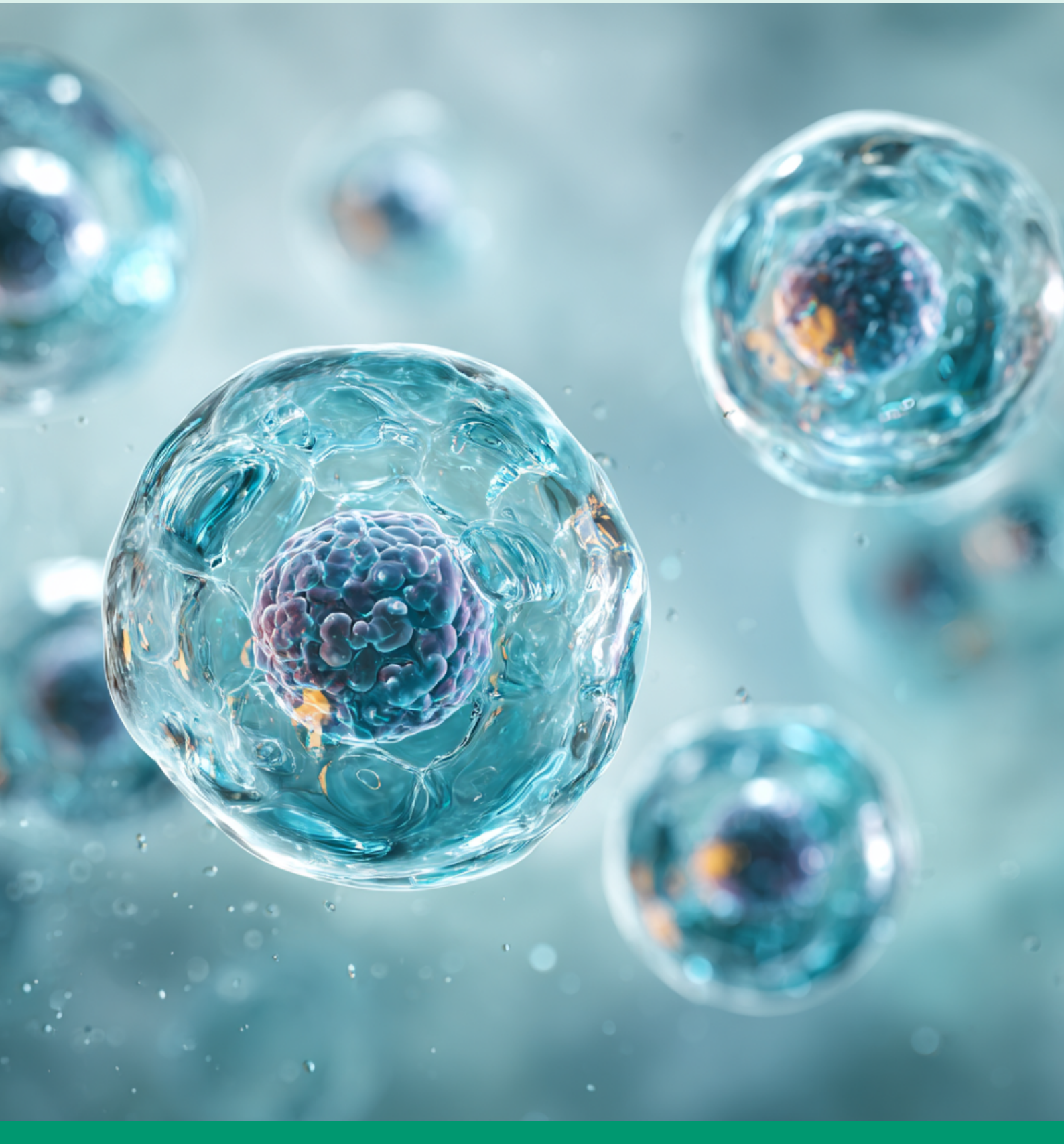
Practical guide to 3D cryopreservation using Bambanker™

Principles, protocols, and best practices for reliable sample preservation



Preface

The ability to store and recover biological material without loss of function is one of the cornerstones of modern bioscience. Advances in cell culture, organoid technology, and tissue-based analysis have expanded the demand for robust cryopreservation methods that are both reliable in outcome and practical in application. Yet, despite decades of use, cryopreservation remains a critical step where variability and sample loss can compromise downstream research and clinical outcomes.

This e-book was created to provide researchers, laboratory professionals, and students with a practical guide to the cryopreservation of 3D biological systems such as organoids, spheroids, and tissue fragments. It combines the fundamental principles of cryobiology with step-by-step workflows, troubleshooting advice, and case examples from recent literature.

Bambanker™ is used here as the reference cryopreservation medium because of its serum-free formulation, ease of use, and proven compatibility with complex biological systems. The aim, however, is not only to describe a product but to illustrate the principles that underlie successful preservation of delicate structures. By focusing on both the “why” and the “how,” this guide is designed to support reproducibility, scalability, and translational research across many fields.

We hope this e-book serves as a useful reference in the laboratory, whether you are establishing new cryopreservation routines, optimizing existing protocols, or preparing samples for advanced downstream applications such as histology, omics, or transplantation studies.

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I. Introduction to cryopreservation

Cryopreservation is a way to pause biological systems at a defined point in time. By lowering the temperature to halt metabolic and enzymatic activity, researchers can preserve the structural and functional integrity of living materials for future use. In doing so, cryopreservation safeguards the complexity of cells, tissues, and organoids so that they remain available for later analysis, experimentation, or therapeutic application.

The central challenge is ice formation. If ice crystals form inside cells, they can rupture membranes and damage organelles. Effective protocols are designed to prevent this by promoting extracellular ice formation while protecting intracellular structures with chemical cryoprotectants. When done correctly, the biological architecture, and the information it carries, can be maintained for months or even years.

This guide provides a structured overview of how cryopreservation can be applied to 3D biological systems such as organoids, spheroids, and tissue fragments. It focuses on practical workflows and scientific principles, while highlighting how Bambanker™, a ready-to-use, serum-free cryoprotectant, can simplify these processes.

Whether the goal is to bank stem cell–derived neurospheres for transplantation, to freeze epithelial organoids for histological analysis, or to store tissue for spatial omics, the principles remain the same: minimize structural damage, maintain viability, and ensure reproducibility. By combining established cryobiological strategies with a reliable medium, researchers can approach cryopreservation with both confidence and consistency.

Key takeaway:

- Cryopreservation halts biological processes by reducing temperature to near standstill.
- The main challenge is preventing intracellular ice formation, which damages cells and tissues.
- Cryoprotectants such as Bambanker™ enable the safe storage and later recovery of cells, organoids, and tissues.

II. Why use Bambanker™?

Choosing a cryopreservation medium for tissues, organoids, and other 3D structures involves different priorities than single-cell freezing. In 3D systems, post-thaw success is strongly influenced by preservation of architecture, consistent cryoprotectant exposure across the structure, and standardized handling that reduces variability between users and laboratories.

Bambanker™ is a ready-to-use, serum-free cryopreservation medium formulated with stabilizing components that are intended to minimize ice-related damage during freezing and thawing. From

a workflow perspective, it enables a simplified process in which samples can be resuspended and stored at
−80 °C without requiring controlled-rate freezing equipment. This can be particularly useful for routine laboratory settings or multi-user environments where protocol consistency is important.

For 3D cultures, practicality and reproducibility matter as much as the medium itself. Organoids and tissue fragments are sensitive to mechanical stress and to uneven penetration of cryoprotectants, especially when extracellular matrix is present. A standardized medium supports consistent processing, while tissue-appropriate handling (e.g., gentle pipetting, matrix removal where applicable, and optimized recovery media) remains essential for maintaining morphology and function after thaw.

Finally, in translational and regulated contexts, standardized materials and documentation become more critical. Because Bambanker™ formulations are GMP-compliant and serum-free, they support more standardized workflows and can reduce variability introduced by serum-containing formulations.

Key takeaway:

- Tissue and organoid cryopreservation requires preservation of 3D architecture and consistent handling.
- Bambanker™ is a serum-free, ready-to-use cryopreservation medium designed for standardized workflows across users and laboratories
- Direct storage at −80 °C can simplify routine cryopreservation without controlled-rate freezing equipment.
- Post-thaw outcomes still depend on tissue-appropriate handling and recovery conditions.

III. Applications and examples from literature

Published studies have used Bambanker™ to biobank a range of 3D biological samples—including intact neurospheres and organoids, organoid-derived cells, and native tissues for downstream workflows such as re-culture and expansion, histology/IHC, and nuclei-based multi-omics. The examples below summarize (i) what was cryopreserved, (ii) how Bambanker™ was used in the workflow, (iii) the downstream application after thaw, and (iv) any handling notes explicitly stated by the authors (Table 1).

Table 1: Cryopreservation protocols, and outcomes using Bambanker™.

Sample	Study	Protocol highlight	Outcome
iPSC Neurospheres	Yin Y. *et al.*, (2025)	3D cryopreservation, Proton Freezer	TH+ expression retained, viable growth
NEPC patient-derived organoids & murine prostate organoids	Singh N. *et al.*, (2020) Singh N. *et al.*, (2021)	Organoid cultures biobanked using Bambanker™ (−80 °C); maintained in Matrigel-based organoid culture	Enabled ongoing organoid-based downstream experiments
Mouse intestinal tumor–derived organoids	Morita A. *et al.*, (2024)	Bambanker™ frozen stock → recovery in 3D Matrigel; spleen transplantation; IVIS luciferase monitoring; histology/IHC	Recovered organoids enabled *in vivo* liver metastasis modeling and downstream tissue analysis
Cardiac tissue	Qaqorh T. *et al.*, (2025)	snRNA-seq, spatial transcriptomics after freezing	Preserved nuclei, clear expression states
Brain tissue	Pokhrel N. *et al.*, (2025)	Multiomics, nuclei extraction after Bambanker™ preservation.	Intact nuclei, suitable for sequencing

Note: Outcomes reflect each study's reported downstream readout and are not directly cross-comparable across models and assays.

Dopaminergic neurospheres (Yin Y. *et al.*, 2025; Hiramatsu S. *et al.*, 2021)

- **Sample:** Induced pluripotent stem cell (iPSC)-derived dopaminergic neurospheres.
- **Protocol:** Neurospheres were cryopreserved in Bambanker™ hRM and stored using a Proton Freezer. Samples were frozen intact (not dissociated), emphasizing the preservation of 3D architecture.
- **Outcome:** High post-thaw viability and neurite outgrowth; preserved tyrosine hydroxylase (TH) expression, confirming dopaminergic identity (Figure 1).
- **Special considerations:** Avoid dissociation and mechanical disruption; use low-speed centrifugation and neurotrophic supplements post-thaw.

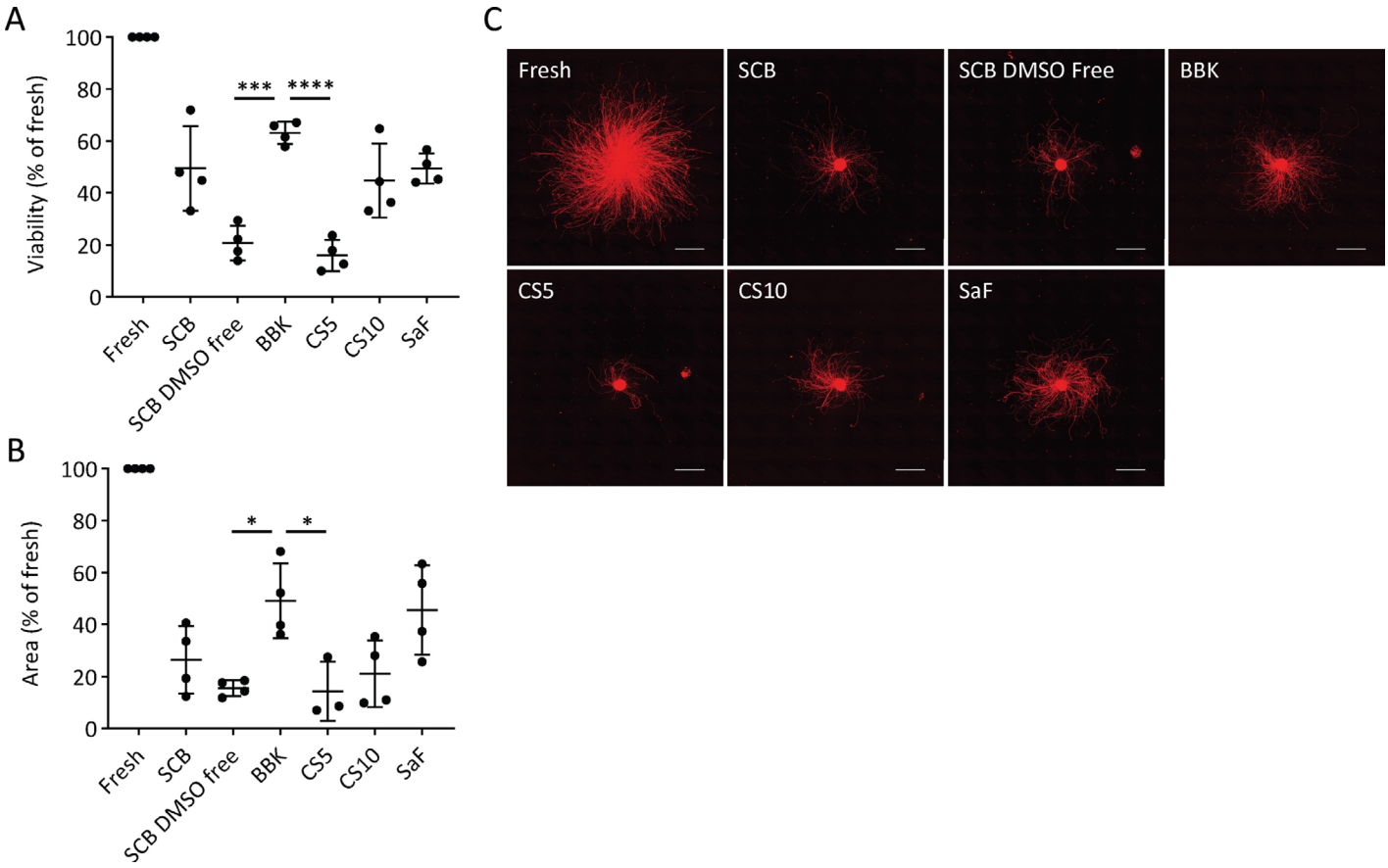


Figure 1: Effects of cryopreservation media on iPSC-derived neurospheres.
(A) - Viability and (B) - neurite extension of spheres from unsorted cells cryopreserved at −0.5°C/min on day 28 using the cryopreservation media shown in table 1 (n=4). Viability and neurite extensions were analyzed on day 1 (24 hours) and day 5, respectively. One-way ANOVA with Tukey's multiple comparisons test; *p<0.05, ***p<0.001, ****p<0.0001 *versus* Bambanker hRM.
(C) - Immunostaining of neurites for PSA-NCAM. Scale bars, 1mm. Data are shown as means±SD.
SCB - STEM-CELLBANKER | BBK- Bambanker™ | CS5 - Cryostor CS5 | CS10 - Cryostor CS10 | SaF- Synth-a-Freeze
Reproduced from Hiramatsu et al., Journal of Parkinson's Disease 12(3), 871–884 (2022), DOI:10.3233/JPD-212934 (Ref. X), licensed under CC BY 4.0.

NEPC patient-derived organoids & murine organoids (Singh N. *et al.*, 2020; Singh N. *et al.*, 2021)

- **Sample:** NEPC patient-derived organoids OWCM-154, OWCM-155, OWCM-1078, and OWCM-1262 lines & murine organoids derived from wild-type mouse prostate cells
- **Protocol:** Dense organoid cultures (200–500 µm organoids) were directly biobanked at −80 °C using Bambanker™.
- **Outcome:** Organoid cultures were biobanked in Bambanker™ at −80 °C as part of routine maintenance of the model, enabling preservation of organoid lines for later use. Post-thaw recovery metrics were not reported.
- **Special considerations:** Both NEPC and murine organoids were replenished with fresh media every 3–4 days during organoid growth.

Mouse intestinal tumor–derived organoids (Morita A. *et al.*, 2024)

- **Sample:** Genotype-defined intestinal tumor organoid lines (early- and late-stage adenocarcinoma models; e.g., AP, AK, AKF), cryobanked as organoid-derived cells (cell pellet) in Bambanker™.
- **Protocol:** Organoid cell pellet resuspended in 500 µL Bambanker™ (~3–5 × 10^5 cells/tube),

frozen overnight at −80 °C, then stored in liquid nitrogen. Thaw at 37 °C, wash at 300×g, re-embed in Matrigel (30 µL/well, 48-well plate) and recover in 3D medium.

- **Outcome:** A complete workflow is described to generate frozen tumor-organoid stocks using Bambanker™ and recover them back into 3D Matrigel culture for continued expansion. The recovered organoids can then be used for downstream applications including spleen transplantation to model liver metastasis, IVIS luciferase bioluminescence monitoring, and histology/immunohistochemistry.
- **Special considerations:** Pre-coat tips with FBS to reduce organoid loss; avoid bubbles in Matrigel suspensions; genotype/handling-dependent behavior noted; reconstitution of 3D organoids from dissociated cells described.

Neural tissue (Pokhrel N. *et al.*, 2025)

- **Sample:** Rat brain tissue for multiomic analysis (ATAC-seq and RNA-seq).
- **Protocol:** Brain tissue was frozen with Bambanker™ and processed using a gentle nuclei isolation method.
- **Outcome:** Intact nuclei with minimal debris; suitable for integrated chromatin and transcriptomic analysis (Figure 2).
- **Special considerations:** Maintain cold chain rigorously; avoid mechanical overlysis during homogenization.

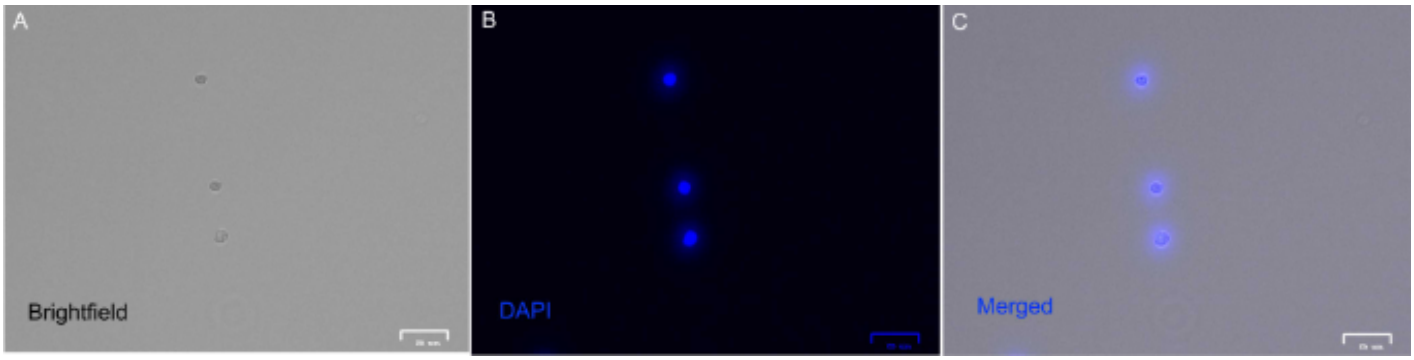


Figure 2: Evaluating the quality of nuclei for single-cell multiome analysis.
Nuclei with intact round morphology are regarded as high quality and are optimal for single-cell multiome library preparation. (A) - Brigheld image of brain nuclei, (B) - DAPI staining of brain nuclei, (C) - Merged image of brighteld and DAPI channel.
Reproduced from Pokhrel N, Telese F. Nuclei Isolation Protocol Multiome 10X Genomics (protocols.io), Version 1, doi:10.17504/protocols.io.81wgbzd41gpk/v1, Section 7 (Ref. X), licensed under CC BY 4.0.

Cardiac Tissue (Qaqorh T. *et al.*, 2025)

- **Sample:** Mouse ventricular cardiac tissue in a mitochondrial cardiomyopathy model.
- **Protocol:** Tissues were cryopreserved in Bambanker™ and used for single-nucleus RNA sequencing (snRNA-seq) and spatial transcriptomics.
- **Outcome:** High-quality nuclei and intact transcriptomic profiles; spatial heterogeneity and cardiomyocyte states were successfully analyzed (Figure 3).
- **Special considerations:** Keep tissues frozen until nuclei isolation; minimize thaw time to preserve nuclear integrity.

Key takeaway:

- Bambanker™ has been applied across diverse biological contexts, including neurospheres; epithelial, prostate, and intestinal organoids; as well as cardiac and brain tissue.
- Studies show it preserves structural integrity and molecular fidelity, enabling downstream applications such as transcriptomics and multi-omics, transplantation, and IVIS luciferase monitoring.
- Freezing organoids and neurospheres intact rather than dissociated supports higher viability and function.
- Bambanker™'s ability to allow direct −80 °C freezing simplifies workflows and supports reproducibility in both routine laboratories and high-throughput biobanking.

IV. Tips and troubleshooting

Effective cryopreservation of organoids and tissues depends on controlling small variables across the entire freeze–thaw cycle. In 3D systems, outcomes are often determined by how gently structures are handled, how uniformly the cryoprotectant contacts the sample, how fast thawing is performed, and how quickly the cryoprotectant is diluted or removed after thawing.

Prevent clumping and mechanical damage. Aggregation is common in spheres and organoids, especially when residual extracellular matrix is present. Minimize shear (no vortexing), keep pipetting to the minimum needed to achieve a uniform suspension, and use low-stress handling throughout. Where matrix must be removed as part of processing, enzymatic release and gentle pelleting have been used in epithelial organoid workflows to preserve structure during downstream preparation (Lee *et al.*, 2025).

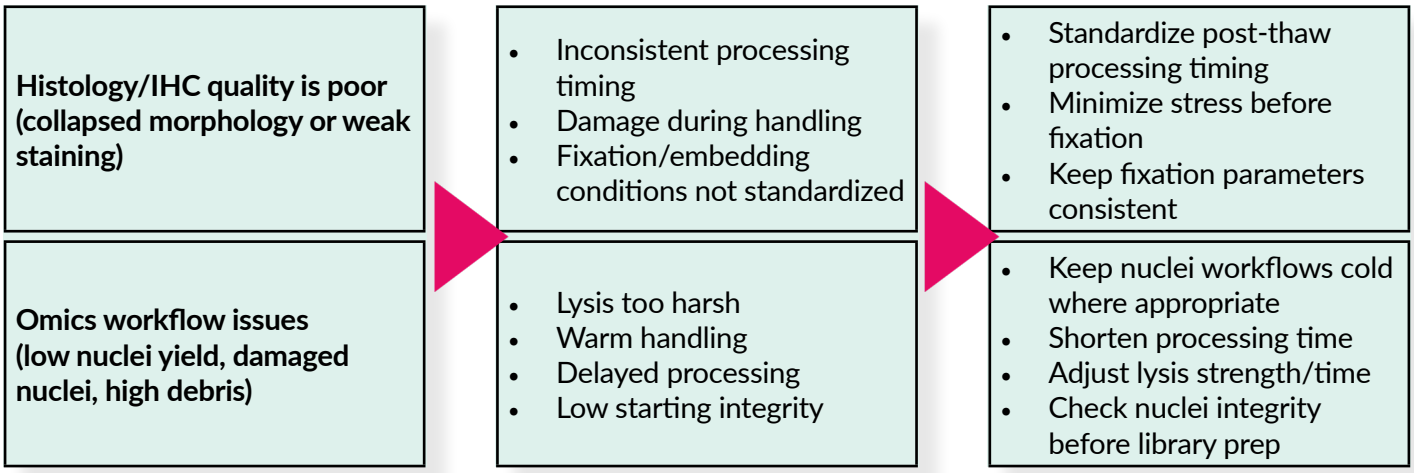
Limit post-thaw cryoprotectant exposure. DMSO-based cryoprotectants are effective during freezing, but prolonged exposure after thawing can reduce recovery. Plan the thaw so samples are promptly diluted into pre-warmed recovery medium and processed without delays. In an epithelial organoid workflow, cryopreserved samples were rapidly thawed at 37 °C and washed before downstream fixation/embedding, illustrating the principle of rapid thaw followed by prompt cryoprotectant removal (Lee *et al.*, 2025). In a tumor organoid protocol, thawing was followed by wash steps (including pelleting) before returning material to 3D culture, reinforcing the same recovery logic for organoid handling (Morita *et al.*, 2024).

Optimize recovery conditions to the biology. There is no universal recovery medium for 3D cultures. Use tissue-appropriate supplements and consider adding supportive factors during the first 24–48 hours to stabilize morphology and function (follow your established organoid/neurosphere culture requirements).

Use morphology as the first QC readout. Early post-thaw assessment should include morphology, not only viability counts. In neural sphere models, post-thaw outgrowth and retention of lineage

markers provide a fast functional checkpoint after cryopreservation (Yin *et al.*, 2025). For epithelial organoid workflows intended for histology/IHC, preservation of architecture and antigenicity after cryopreservation and processing supports morphology/IHC as an appropriate downstream quality readout (Lee *et al.*, 2025). For omics-oriented workflows, maintaining cold handling during nuclei preparation is emphasized to protect nuclei integrity and reduce technical artifacts (Qaqorh *et al.*, 2025; Pokhrel *et al.*, 2025). For consistency across experiments, freeze organoids from a comparable culture state (e.g., similar size/density and passage timing), which aligns with studies that used Bambanker™ for routine, repeated biobanking as part of ongoing organoid maintenance (Singh *et al.*, 2020; Singh *et al.*, 2021).

What you observe	Likely reason	What to try next
Severe clumping immediately after thaw	• Matrix carryover • Delayed dilution/removal • Harsh mixing; over-dense vial	• Dilute promptly into warm medium • Add a gentle wash step if appropriate • Standardize matrix handling • Reduce load per vial to minimize shear stress
Structures look "shredded"/ fragmented	• Excess mechanical stress during transfer • Harsh centrifugation • Inconsistent thaw timing	• Use gentle pipetting (low shear) • Reduce centrifugation force/time • Avoid vigorous resuspension • Standardize thaw workflow
Looks OK at 24 h, but poor regrowth by Day 3–7	• Suboptimal recovery conditions • Prolonged post-thaw exposure • Banked from stressed/ overgrown cultures • Size too large	• Optimize recovery medium • Shorten time-to-dilution/ removal • Bank from a consistent healthy state • Standardize size-window banking (don't freeze a mixed population of tiny + very large structures)
High debris/low viable singlets after dissociation	• Over-dissociation • Prolonged processing • High cell death during handling	• Shorten dissociation time • Use gentler conditions • Keep timing consistent • Report viable singlets and doublet rate
Loss of expected cell types/ marker drift after recovery	• Selective loss of sensitive populations • Inconsistent banking state • Recovery conditions driving phenotype changes	• Bank from standardized culture state • Confirm identity using 2–4 markers • Adjust recovery conditions to maintain intended phenotype



Key takeaway:

- Outcomes in 3D cryopreservation are sensitive to small variables across the freeze–thaw cycle, including handling intensity, cryoprotectant exposure, thaw speed, and post-thaw processing.
- Reduce aggregation and mechanical damage by minimizing harsh mixing and using gentle, consistent handling, especially when extracellular matrix is present.
- Plan for rapid thawing and prompt dilution/removal of Bambanker™ to support recovery and reduce post-thaw stress.
- Optimize recovery conditions to the biology of the model; there is no universal recovery medium for all organoids and tissues.
- Use morphology as an early QC readout, and align it with your downstream application (e.g., outgrowth/marker retention, histology/IHC readiness, or omics suitability).

V. QC checklist for cryopreservation of 3D biological samples

(tissues, organoids, spheroids)

Purpose: Verify that cryopreserved 3D samples recover reproducibly after thaw, maintain identity/composition, and remain fit for the intended downstream application (re-culture, histology/IHC, functional assays, or omics).

1) Record what was banked (critical for interpretation)

- ☐ **Sample type:** tissue/organoid/spheroid
- ☐ **Culture state (organoids/spheroids)**: passage number, days since last split, general health notes
- ☐ **Size/load per vial (choose at least one):**
  - organoids/spheroids: estimated number of structures per vial and a typical size band (e.g., 100–200 µm; 200–500 µm)
  - tissue: fragment size category (e.g., ~1–2 mm^3) or mass per vial (mg)
- ☐ **Matrix status:** embedded in Matrigel/BME? Yes/No (and whether matrix was reduced/removed before banking)
- ☐ **Bambanker™ details:** product line + lot number; time from Bambanker™ contact to placement at −80 °C

2) Day 0 (thaw day): immediate recovery QC

Handling + timing (process QC)

- ☐ Record thaw method (e.g., 37 °C water bath) and time-to-dilution/removal (thaw completion → dilution into warm medium/buffer)
- ☐ Record recovery path:
  - organoids/spheroids: re-embedding method (matrix type, dome/volume, plate format)
  - tissue: dissociation to single cells *vs* fragment/explant culture *vs* nuclei preparation (for omics)

Immediate visual QC (5–15 min)

- ☐ Score: intact structures *vs.* fragmentation; clumping/aggregation; debris level
- ☐ **Stop flags (don't proceed without troubleshooting):** severe clumping that prevents handling, extensive fragmentation ("shredded" structures), or long post-thaw delays before dilution/removal

3) Early QC (4–24 h): *"Is it stabilizing?"*

Organoids/spheroids

- [ ] **Morphology:** structures remain cohesive; limited debris; no widespread collapse
- [ ] **Recovery behavior:** re-compaction (spheroids), early re-organization/lumen re-formation (organoids, model-dependent)
- [ ] Optional quick documentation: one representative image per condition (same magnification)

Tissue (fragment/explant culture)

- [ ] **Integrity:** fragment remains cohesive (no excessive disintegration)
- [ ] **Signs of recovery:** attachment/outgrowth (if expected for your model); limited necrotic expansion (if assessable)

If dissociated for analysis

- [ ] Record total yield, viability method, and "viable singlets *vs* aggregates" (aggregation is common post-thaw and affects downstream assays).

4) Growth QC (Day 2–7): *"Is it behaving like the original model?"*

Use at least one quantitative metric that is easy for your lab.

Organoids/spheroids

- [ ] **Regrowth**
  - % structures that re-expand
  - median diameter/area over time
  - number of viable organoids per well after re-plating
  - passaging success (yes/no + time to first split)
- [ ] **Batch consistency:** compare 2–3 vials from the same freeze batch to confirm reproducibility.

Tissue (fragment/explant culture)

- [ ] **Fragment growth/outgrowth** (if applicable), or **yield and composition consistency** (if dissociated).

5) Identity and composition QC (recommended when used as a model system)

Especially important for long-term biobanking, collaborations, or publishable work.

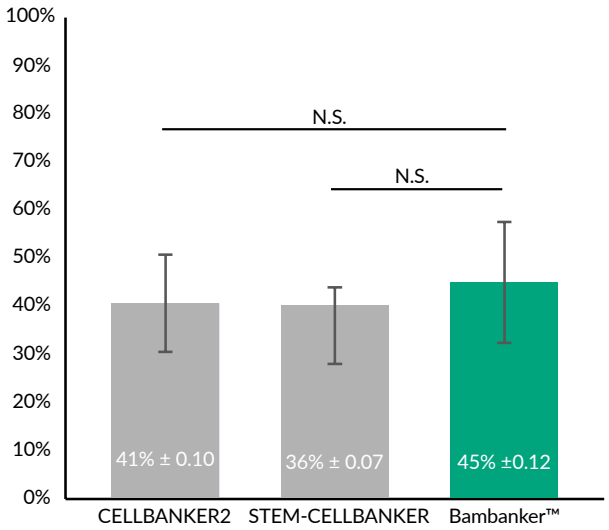
- ☐ **Marker-based QC:** stain or immunolabel 2–4 markers relevant to the model
  - one lineage/identity marker
  - one functional marker
  - optional proliferation marker (e.g., Ki-67) or stress/apoptosis marker (only if informative)
- ☐ **Flow cytometry:** only if the sample is already dissociated; always report viable singlets and doublet rate.

VI. Comparative performance: Bambanker™ *vs.* competitors

The performance of a cryopreservation medium is best understood when it is tested under direct comparison with other available reagents. A recent study assessed Bambanker™ alongside two widely used alternatives — CELLBANKER2 and STEM-CELLBANKER GMP grade — in the context of tissue cryopreservation. The goal was to evaluate not only overall viability but also the preservation of specific immune cell populations, which are often more sensitive to freeze–thaw stress.

Briefly, in this study, spleens were cut into equal pieces and frozen for 10 days at –80°C in each of the three media. Post-thaw analysis using cell counting and flow cytometry revealed that Bambanker™ consistently resulted in the highest survival rates (Figure 1). Specifically, Bambanker™

A. Cell counter



B. Flow cytometry

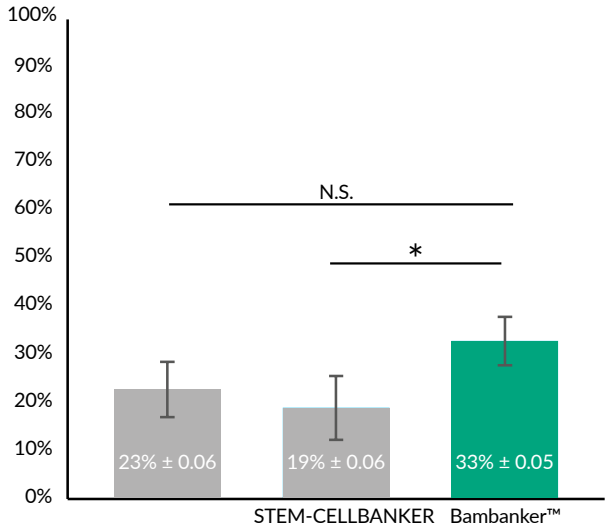
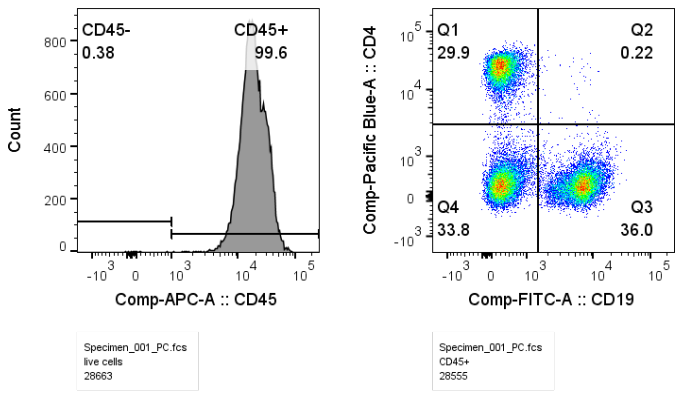


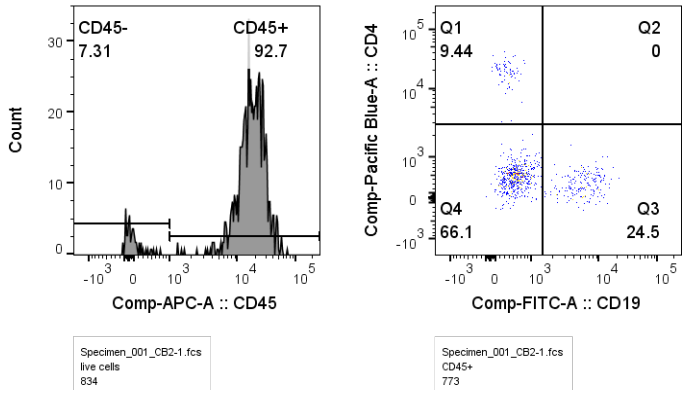
Figure 1: Number of living cells assessed by cell counter (A) and flow cytometry (B) after cryopreservation of mouse spleen tissue in 3 different cryopreservation media. ** p < 0.05 (Student t-test; n= 3) N.S. - Not Significant*

showed 45% ± 0.12 viability (cell counter) and 33% ± 0.05 (flow cytometry), outperforming CELLBANKER2 and STEM-CELLBANKER. Moreover, staining for CD4 and CD19 markers indicated better preservation of T and B lymphocytes in Bambanker™-preserved samples (Figure 2).

Control



CELLBANKER2



STEM-CELLBANKER *Bambanker™*

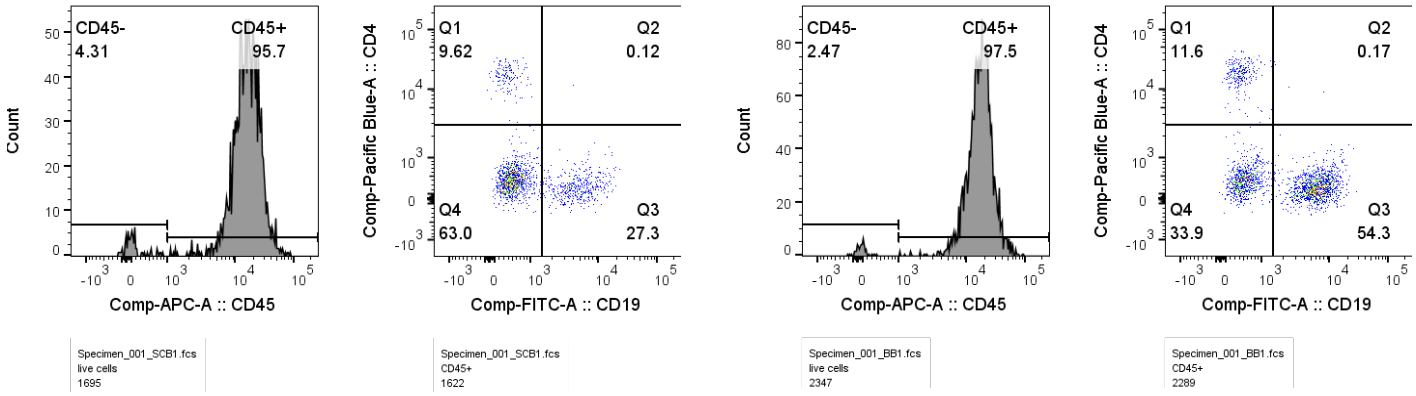


Figure 2: Identification of cells subsets from a mouse spleen after being cryopreserved for 10 days with three different cryopreservation media: CELLBANKER2, STEM-CELLBANKER, and Bambanker™.

Cell surface markers were labeled with with APC anti-mouse CD45, Pacific Blue anti-mouse CD4 and FITC anti-mouse CD19.

In this comparative context, Bambanker™ demonstrated consistent advantages in terms of viability, suspension quality, and immune cell preservation when applied to mouse spleen tissue cryopreservation. These results illustrate its capacity to meet one of the most challenging goals in cryobiology: maintaining the diversity and integrity of immune cells within intact tissue fragments.

For full details, download the full application note: https://www.nippongenetics.eu/app/uploads/AN-BB0-Bambanker-tissue-freezing-competitor-analysis.pdf

Key takeaway:

- Bambanker™ yielded the highest post-thaw viability.
- Samples frozen in Bambanker™ showed fewer aggregates and more single-cell events, improving suitability for downstream immune analyses.
- Immune cell subsets ($CD4^+$ T cells, $CD19^+$ B cells) were better preserved in Bambanker™ compared with competitor reagents.
- The study notes that while thawing and processing can influence outcomes, Bambanker™ consistently supported higher tissue and immune cell recovery under the tested conditions.

VII. Future directions

As biological research advances, the demands placed on cryopreservation are evolving. What was once a technique focused primarily on single cells is now expected to maintain the structural and functional complexity of 3D biological systems such as organoids and intact tissue fragments. These advances open new possibilities for discovery and clinical translation, but they also introduce new technical challenges.

Organoids and screening scalability
Organoids are increasingly being adapted for medium- and high-throughput screening (HTS) in drug discovery, toxicology, and personalized medicine. While they are still more complex to automate than traditional 2D assays, advances in miniaturized culture formats, automated liquid handling, and high-content imaging are enabling organoid assays in standardized multiwell plates, including 96- and 384-well workflows (Czerniecki *et al.*, 2018; Bozal *et al.*, 2024; Du *et al.*, 2020).

In academia, organoids are widely used as physiologically relevant models to study human development and tissue function and to model cancer, inherited disorders, host–pathogen interactions, and patient-specific treatment responses; in neuroscience, brain organoids and region-specific neural spheroids are used to investigate neurodevelopment, cell-type specification, and features of neurological disease, often using patient-derived iPSC models.

As these workflows scale, reproducible biobanking becomes a practical bottleneck: organoid programs need consistent cryostorage so that the same biological model can be recovered across experiments, teams, and sites with minimal variability. In parallel, commercial platforms such as the OrganoPlate® format support more standardized and semi-automated 3D workflows, helping bridge complex biology with screening-compatible infrastructure (Mimetas BV, 2024; Hub Organoids, 2024).

As these systems move toward larger-scale adoption, cryopreservation protocols will need to support uniform recovery and reproducibility across many parallel cultures, including consistent post-thaw morphology, viability, and downstream assay performance. Ready-to-use cryoprotectants such as Bambanker™ can fit well into these workflows by simplifying handling and reducing operator-dependent variability during routine biobanking.

Clinical translation
As organoid and tissue models move closer to clinical applications in regenerative medicine and precision therapy, quality management, reproducibility, and regulatory readiness become central requirements. In practice, this shifts workflows toward defined, serum-free/animal-component–free inputs and stronger documentation, which better supports standardized manufacturing and quality control expectations in GMP-oriented settings. Ensuring long-term stability while maintaining genomic and functional fidelity after cryostorage will remain a key focus area as biobanking and scale-up increase (Dossena M. *et al.*, 2020; Pamies D. *et al.*, 2021; Pamies D. *et al.*, 2024).

Multi-omics and data integrity

Emerging single-cell and spatial omics approaches require cryopreserved samples that maintain molecular fidelity, preserving not only morphology but also transcriptomic and chromatin integrity. Several studies have shown that cryopreserved samples can produce data quality comparable to freshly processed material for many key QC metrics, enabling reliable single-cell and multi-omic profiling when handling and quality control are standardized (Guillaumet-Adkins A. *et al.*, 2017; Wohnhaas T. *et al.*, 2019). Chromatin accessibility profiling has also been successfully performed from cryopreserved material with quality comparable to fresh samples in reported ATAC-seq workflows (Fujiwara S. *et al.*, 2018). Continued optimization of freezing and thawing protocols will support consistent performance in transcriptomic, epigenomic, and proteomic workflows.

Automation and biobanking

Large-scale biobanking is increasingly shaped by automation and digital infrastructure. Modern biorepositories implement standardized operating procedures alongside automated storage and retrieval (including −80 °C systems) to reduce manual handling, limit temperature excursions during retrieval, and improve overall sample integrity; an important contributor to research reproducibility (Linsen L. *et al.*, 2019).

In parallel, biobank digitalization is expanding traceability and process control through informatics: integrated databases and tracking systems can capture key metadata across the biospecimen lifecycle (e.g., pre-analytical processing steps, freeze–thaw cycles, and storage history), while supporting automated aliquoting, routing, and temperature monitoring to reduce avoidable variation (Bukreeva A. S. *et al.*, 2024). As these “smart biobank” approaches mature, the use of AI-enabled analytics and data-centric governance in the evolution of biobanks is becoming an increasingly discussed topic, particularly with regard to access, scale, and the use of linked sample–data resources (Mayrhofer M., 2025).

Within this context, simplified, ready-to-use cryoprotectants can be particularly compatible with automated systems, where consistency, speed, and safety are critical for throughput and reproducibility. Standardized cryopreservation reagents such as Bambanker™ can therefore support scalable biobanking workflows by reducing operator-dependent steps during routine sample preparation and storage, helping maintain more uniform recovery across large sample sets.

Key takeaway:

- Organoids are increasingly being adapted to medium-/high-throughput workflows, supported by advances in miniaturized culture formats, automated liquid handling, and high-content imaging making larger, more standardized organoid studies increasingly feasible.
- As organoid programs scale across teams, sites, and time, consistent cryopreservation and uniform post-thaw recovery are essential for reproducibility in downstream assays.
- Movement toward regenerative medicine and precision therapy increases the need for defined workflows, documentation, and quality management; long-term stability and functional/genomic fidelity after storage will remain key priorities.

- Single-cell, spatial, and multi-omic analyses require cryopreserved samples that preserve transcriptomic and chromatin quality; standardized handling and QC become as important as morphology.
- Biobanks are integrating automated storage/retrieval and digital traceability, increasing demand for standardized, ready-to-use cryopreservation workflows that reduce operator-dependent variability.
- In these evolving workflows, a standardized, ready-to-use cryoprotectant can support scalability by simplifying routine biobanking and helping maintain consistent handling across high sample volumes.

VIII. Conclusions

Cryopreservation of 3D biological samples such as tissues, organoids and spheroids requires more than preserving viability; it requires preserving structure, function, and molecular integrity in a way that supports reproducible downstream use. Across the workflows discussed in this guide, consistent outcomes depend on controlling key variables across the freeze–thaw cycle: gentle handling of 3D material, appropriate matrix management, rapid and standardized thawing, and recovery conditions matched to the biology of the sample and the intended assay.

Bambanker™ is used across multiple published contexts as a practical cryopreservation medium for complex 3D systems, supporting routine biobanking workflows and downstream applications ranging from culture re-expansion and histology/IHC to functional *in vivo* studies and omics-oriented processing. While individual protocols still require adaptation to tissue type and experimental goals, the overarching value of a standardized, ready-to-use cryoprotectant is the ability to reduce operator-dependent variability and improve consistency across experiments, teams, and storage time.

As organoid and tissue models continue to expand into screening, biobanking, and translational applications, the importance of robust cryostorage will only increase. Taking practical steps such as applying a structured workflow, documenting key handling parameters and implementing simple post-thaw QC (morphology, relevant markers and assay-specific readouts) helps to ensure that cryopreserved samples remain reliable biological models and are ready for the next experiment, analysis or application.

IX. References

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+49 2421 55496 0
+49 2421 55496 11
info@nippongenetics.eu
www.nippongenetics.eu