Bambanker™ Cryopreservation in Cancer Research. A Literature Review.

Product	Bambanker™ (BB05, BB02, BB03, BB04)
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Manufacturer	GC Lymphotec Inc.

This application note was created by the manufacturer: NIPPON Genetics EUROPE GmbH

Purpose

Cancer research relies heavily on the ability to preserve viable cells and tissues for long-term study, reproducibility, and therapeutic development. Reliable cryopreservation is critical for maintaining the integrity of tumor samples, patient-derived models, and functional assays. Bambanker™, a serum-free, ready-to-use cryopreservation medium, has been increasingly adopted in oncology research studies due to its ease of use, high post-thaw viability, and compatibility with a wide range of sample types.

This application note compiles key findings from peer-reviewed publications that employed Bambanker™ to cryopreserve cancer cells and tissues, including tumor fragments, cell suspensions, organoids, and stromal components. By summarizing how Bambanker™ supports cell viability and experimental fidelity across diverse cancer research applications, this note aims to inform and guide researchers in selecting effective preservation strategies.

Literature Review

In this section we outline each study's approach to using Bambanker™ in cryopreserving cancer-related materials. The individual examples demonstrate Bambanker's successful application across a range of cancer models and downstream applications. Table 1 on Page - 6 - summarizes the literature for quick reference and comparison, highlighting the types of samples cryopreserved and the downstream applications performed post-thaw.

In the **Saito et al. (2025)** study, surgically excised brain and ovarian tumor tissues were either kept refrigerated short-term or cryopreserved. Tissues destined for freezing were placed in Bambanker™ medium and stored at −80 °C. Upon experimental use, the tissues were thawed (typically by gentle warming), dissociated into single-cell suspensions, and resuspended in appropriate culture media. These thawed cells were then transfected with NanoLuc reporter constructs to perform the ultra-rapid HR (homologous recombination) and MMR (mismatch repair) assays.

In **Yost et al. (2025)**, the authors report that primary tumor nuclei were cryopreserved in Bambanker™. Specifically, nuclei from 69 tumor samples spanning 15 cancer types (TCGA samples) were isolated and frozen in Bambanker™ for later analysis. One million of these cryopreserved nuclei were thawed per experiment and directly used in HiChIP assays. The paper does not detail a special thaw protocol, but the Methods note that thawed nuclei were subjected to chromatin digestion (with MboI) and sonication (Covaris) as part of an H3K27ac-targeted HiChIP protocol. After thawing, the nuclei were processed for H3K27ac HiChIP to map 3D chromatin contacts. The immunoprecipitated fragments (using an H3K27ac antibody) were used to prepare sequencing libraries, which were then sequenced on an Illumina HiSeq. The resulting data enabled identification of enhancer–promoter loops and other 3D genomic features in the primary cancer samples.

Restivo et al. (2022) used Bambanker™ to cryopreserve small pieces (~2–3 mm^3) of human tumor tissues, including melanoma, colorectal, basal cell carcinoma, and breast cancer. Tissue was slow-frozen at −80 °C and later stored in liquid nitrogen.

Thawing was performed by rapid warming (37 °C water bath), followed by enzymatic dissociation for single-cell recovery. Thawed tissues showed high viability.

Post-thaw, samples were used for 2D cell culture, 3D organoid generation, ex vivo explant culture, and single-cell RNA sequencing (scRNA-seq). Results from frozen samples were highly comparable to fresh tissue in terms of viability, cell type diversity, and gene expression.

Yasuda et al. (2021) developed a method to isolate cancer-associated fibroblasts (CAFs) from gastric cancer tissues. After initial expansion, fibroblasts were frozen using Bambanker™ medium (~$6×10^5$ cells per vial). Samples were slow-frozen at −80 °C and stored

for up to six months.

Thawing was performed by rapid warming (37 °C water bath), followed by centrifugation to remove Bambanker™. Cells were then replated and cultured normally without special coatings.

Post-thaw, fibroblasts were used for downstream experiments: they were exposed to inflammatory cytokines (IL-1α, IL-1β, TNF-α) to induce senescence, mimicking the tumor microenvironment. Senescence was validated by morphology, β-galactosidase staining, and upregulation of p16^INK4a, p15^INK4b, and γH2AX. CAFs showed stronger senescence responses than normal fibroblasts.

Saito et al. (2022) used Bambanker™ to preserve dissociated gastric tumor cells for later immune analysis. Fresh human gastric cancer tissues were cut into small pieces and enzymatically dissociated into single-cell suspensions (referred to as fresh tumor digests, FTDs). The cells were passed through a 70 µm strainer and immediately cryopreserved in Bambanker™ freezing medium until further analysis

For downstream analysis, the frozen FTD samples were quickly thawed in RPMI culture medium. After thawing, cell viability was checked with Zombie Aqua™ fixable viability dye, and the cells were stained with antibodies for immune markers such as CD45, CD3, CD4, CD8, CD14, CD19, and CD56. The post-thaw cells were then analyzed by flow cytometry to profile the tumor microenvironment's immune cells. In addition, some thawed cells were stimulated with PMA/ionomycin (with brefeldin A) prior to staining, allowing assessment of functional cytokine responses (intracellular IFN-γ, TNF-α, IL-2 production) by flow cytometry.

In Hu et al. (2024), patient-derived gastric tumor samples – including primary gastric tumors and their ovarian metastatic lesions – were preserved using Bambanker™ to maintain cell viability for later analysis. Small tissue fragments (~2–3 mm^3) from each tumor were frozen in Bambanker™ at –80 °C. When needed, frozen samples were rapidly thawed (in a 37 °C water bath) and immediately transferred into ice-cold medium to dilute out DMSO. Thawed tissue pieces were then cut into smaller bits and enzymatically dissociated into single cells, yielding viable cell suspensions for downstream experiments.

Post-thaw, the dissociated cells were used for single-cell RNA sequencing, revealing estrogen-responsive fibroblasts in ovarian metastases. Viable fibroblasts cultured from thawed samples were treated with estrogen, which induced MDK secretion. Conditioned media promoted gastric cancer cell invasion, and in vivo models confirmed that estrogen enhanced ovarian metastasis. Bambanker™ enabled high-viability preservation for both sequencing and functional studies.

Sato et al. (2015) developed a reproducible CYP3A4 inhibition assay using cells cryopreserved in Bambanker™. They infected HepG2 liver carcinoma cells with an adenoviral vector carrying the human CYP3A4 gene, then suspended the cells in Bambanker™ cryopreservation medium and stored them in liquid nitrogen. This created a frozen stock of CYP3A4-expressing HepG2 cells (termed fAd-CYP3A4 cells) that could be thawed on demand for experiments. The frozen cells were thawed quickly in a 37 °C water bath and washed with fresh culture medium to remove the cryoprotectant before use.

Post-thaw, the recovered cells were plated and used for various CYP3A4 assays. The researchers measured CYP3A4 enzyme activity using a luciferin-based luminescent assay and also assessed CYP3A4 gene expression (via qPCR) and protein levels (via immunoblot). They then examined how known CYP3A4 inhibitors (like ketoconazole) and several natural herbal extracts affected the enzyme activity in these thawed cells. Notably, the cryopreserved cells retained consistent CYP3A4 activity (comparable to fresh cells) even after long-term storage, and the inhibitor potency observed in the thawed cells was equivalent to that in fresh cells or microsomes. This

demonstrated that Bambanker™-frozen HepG2/CYP3A4 cells can reliably be used to evaluate CYP3A4 inhibition by natural medicines.

Przytupski et al. (2019) cryopreserved SKOV-3 human ovarian cancer cells (an established ovarian carcinoma line) using Bambanker™. Approximately 1×10^5 SKOV-3 cells were suspended in Bambanker™ per 50 µL aliquot and frozen for further analysis.
Thawing was performed by rapidly warming the frozen cell aliquots (e.g. in a 37 °C water bath) and immediately diluting into cold PBS. The cells were then pelleted by centrifugation (10 min at 4 °C, ~6,700×g) to remove the Bambanker™ solution. After this wash, the recovered SKOV-3 cells were resuspended in fresh medium for experiments. Post-thaw, the cells were used in downstream assays – notably a neutral comet assay (single-cell gel electrophoresis) to evaluate DNA damage. The thawed SKOV-3 cells were embedded in low-melting agarose on slides and subjected to lysis and electrophoresis; stained comets (nuclei with DNA migration) were then analyzed to quantify DNA damage (tail DNA percentage). This demonstrates that Bambanker™-preserved SKOV-3 cancer cells can be thawed and immediately used for functional assays like DNA damage analysis.

In Barnes et al. (2021) Bambanker™ was used to freeze viable cancer-derived cells for long-term storage. In one study, freshly isolated ovarian cancer cells (from patient ascites or dissociated tumor tissue) and their accompanying stromal fibroblasts were cryopreserved in Bambanker™. After storage in liquid nitrogen, these cells were thawed and directly placed into culture using specialized ovarian cancer medium (OCMI with serum) to resume growth.
Post-thaw, the recovered cancer cells and tissue samples were fully viable, enabling downstream experiments. For the ovarian cancer cells, researchers established ex vivo cell cultures and then performed bulk RNA sequencing (RNA-seq) to profile gene expression. This allowed them to classify the models into ovarian cancer subtypes using machine-learning approaches.

Seth et al. (2019) established clonal pancreatic tumor cell populations and used Bambanker™ cryomedium to preserve a portion of these cells during their lineage-tracing experiments. Specifically, patient-derived pancreatic tumor cells (barcoded to track subclones) were expanded in vitro to an early passage, then split – one fraction was cryopreserved in Bambanker™ and stored (liquid nitrogen) while the other continued in culture. The cryopreservation was done without a programmable freezer, demonstrating Bambanker™'s suitability for one-step freezing.
After storage, thawing was performed by rapidly warming the cells and re-culturing them. For example, frozen clonal cells in 96-well plates were thawed by directly adding warm (37 °C) medium to each well. Post-thaw, the cells were viable and used for downstream clonal expansion and functional assays. The authors could regrow these thawed tumor cell clones and perform in vivo transplantation into mice to form "clonal replica tumors," as well as in vitro analyses.

Singh et al. (2021) developed 3D prostate cancer organoid models of treatment-induced neuroendocrine prostate cancer (NEPC) and used Bambanker™ to cryopreserve these organoid cultures for. Patient-derived and mouse-derived prostate organoids were grown in Matrigel with defined growth factors, and once organoid colonies became dense (200–500 µm), they were harvested and trypsinized into smaller cell clusters for freezing. The organoid fragments were then frozen in Bambanker™ and stored at –80 °C (short-term) before transfer to liquid nitrogen. This allowed the authors to bio-bank NEPC organoids at various stages without loss of viability.

For recovery, frozen organoid vials were rapidly thawed (e.g. in a 37 °C water bath) and the cells were re-plated in fresh Matrigel drops with organoid culture medium. The thawed organoids resumed growth readily, forming viable organoid cultures. These post-thaw organoids were then utilized in downstream experiments. In this study, a key application was the generation of organoid-derived xenografts: the authors injected ~5×10^5 thawed organoid cells (mixed 1:1 with Matrigel) subcutaneously into immunodeficient mice. The resulting tumors were used to examine NEPC behavior in vivo. Additionally, part of each tumor was processed for histological and molecular analyses – formalin-fixed for immunohistochemistry and fresh-frozen for RNA extraction.

Chen et al. (2023) presented a protocol for testing an immunotherapeutic peptide in syngeneic orthotopic glioma models (murine glioblastoma). As part of this workflow, they used Bambanker™ to cryopreserve single-cell suspensions from mouse brain tumors for batched analyses. Specifically, after extracting and enzymatically dissociating brain tumors from treated mice, the authors could pause the experiment and freeze the tumor cells in Bambanker™, storing them in liquid nitrogen for up to 2 months. Multiple tumor samples could be accumulated and later analyzed together to ensure consistency. Tumor cells were frozen in Bambanker™ without needing gradual cooling, highlighting a convenient preservation method for delicate primary glioma cells.
When ready for analysis, glioma cells were rapidly warmed and the cells gently pelleted to remove cryoprotectant. The thawed cells were immediately placed in ice-cold FACS buffer and prepared for flow cytometry staining. Post-thaw viability was high, enabling the authors to proceed with flow cytometric analyses of immune and tumor cell populations.

Martínez-Sabadell et al. (2022) developed a humanized mouse model of acquired resistance to immunotherapy using patient-derived xenografts (PDXs). They incorporated Bambanker™ to safeguard these unique tumor samples throughout the multistage in vivo process. Fresh patient tumor biopsies (breast, pancreatic, colorectal, or gastric cancers) were first implanted into NSG mice to establish baseline PDX tumors. The protocol notes that biopsies can be frozen in Bambanker™ before implantation if necessary, although fresh implantation is preferred for maximum engraftment success. Once the PDX tumors were growing and subjected to cyclic immunotherapy (e.g., T cell bispecific antibodies or CAR T cells), the team passaged the tumors to new mice to evolve resistance. At each passage, a fraction of the tumor was cryopreserved in Bambanker™ as a tumor cell pellet, creating a frozen archive at that timepoint. This means after every round of treatment and tumor regrowth, some tumor tissue was saved in Bambanker™ to ensure the model could be recovered later if needed. Thawed PDX samples were used to re-establish tumors in mice, demonstrating that Bambanker™-preserved tumor pieces remained viable for engraftment.

Table 1: Summary of publications with Bambanker™ used for cryopreservation in cancer research.

Study	Sample Type	Cancer Type	Applications	Title	PubMed ID
Saito et al. 2025	Patient tumor tissue pieces (brain, ovarian)	Brain, ovarian	HR/MMR functional assays	HR eye & MMR eye: one-day assessment of DNA repair-defective tumors eligible for targeted therapy	40355434
Yost et al. 2025	Primary tumor nuclei	15 TCGA cancer types (e.g. colorectal, liver, etc.)	3D enhancer–promoter mapping	Three-dimensional genome landscape of primary human cancers	40355593
Restivo et al. 2022	Small pieces of human tumor tissue (~2–3 mm^3) frozen in Bambanker cryopreservation medium	Multiple (melanoma, colorectal carcinoma, basal cell carcinoma; also breast cancer tissue)	2D cell culture; 3D organoid culture; ex vivo tissue culture; single-cell RNA sequencing	Live slow-frozen human tumor tissues viable for 2D, 3D, ex vivo cultures and single-cell RNAseq	36307545
Yasuda et al., 2021	Primary CAFs (fibroblasts from gastric tumor tissue) frozen in Bambanker	Gastric cancer	Culture expansion; cytokine-induced senescence; senescence assays	Protocol to establish cancer-associated fibroblasts from surgically resected tissues and generate senescent fibroblasts	34136831
Saito et al., 2022	Tumor digest (dissociated tumor cells) frozen in Bambanker	Gastric cancer	Immune profiling and cytokine assays via flow cytometry	Selection of RNA-based evaluation methods for tumor microenvironment by comparing with histochemical and flow cytometric analyses in gastric cancer	35595859
Hu et al., 2024	~3 mm^3 tumor tissue pieces (patient-derived gastric tumor and ovarian metastasis specimens)	Gastric cancer (ovarian metastases)	Single-cell RNA sequencing of tumor microenvironment; primary fibroblast culture and estrogen stimulation assay; cancer cell migration/invasion assays; in vivo metastasis model	The estrogen response in fibroblasts promotes ovarian metastases of gastric cancer	39349474
Sato et al. 2015	HepG2 cells (adenovirus-transduced to express CYP3A4)	Liver (hepatocellular carcinoma)	CYP3A4 activity inhibition assay (luciferin-based), gene expression & protein analysis	Development of a highly reproducible system to evaluate inhibition of cytochrome P450 3A4 activity by natural medicines	26626238
Przystupski et al. 2019	SKOV-3 ovarian cancer cells (cryopreserved suspension)	Ovarian cancer (cell line)	Neutral comet assay for DNA damage analysis after thawing (post-treatment)	The Cytoprotective Role of Antioxidants in Mammalian Cells Under Rapidly Varying UV Conditions During Stratospheric Balloon Campaign	31427965
Barnes et al., 2021	Patient-derived ovarian cancer cells and stromal cells (from ascites or tumors) frozen in Bambanker	Ovarian cancer	Ex vivo culture expansion and bulk RNA-seq for transcriptional subtype analysis	Distinct transcriptional programs stratify ovarian cancer cell lines into the five major histological subtypes	34470661
Seth et al. 2019	Patient-derived pancreatic tumor cells (clonal, barcoded) – frozen at early passage (P2)	Pancreatic cancer (PDAC)	In vitro regrowth of clones; in vivo tumor formation (clonal xenografts); lineage tracing and chemoresistance analysis after thaw	Pre-existing Functional Heterogeneity of Tumorigenic Compartment as the Origin of Chemoresistance in Pancreatic Tumors	30726735
Singh et al. 2021	NEPC prostate organoids (patient-derived and mouse) – frozen as organoid fragments (post-passage)	Prostate cancer (NEPC subtype)	Revival and expansion of organoids; formation of organoid-derived xenograft tumors in mice; molecular assays on tumors (IHC, RNA-seq) post-thaw	The long noncoding RNA H19 regulates tumor plasticity in neuroendocrine prostate cancer	34934057
Chen et al. 2023	Murine glioblastoma tumor cells (from brain of orthotopic GL261 gliomas) – frozen as single-cell suspensions	Glioma (mouse GBM model)	Flow cytometry analysis of tumor-infiltrating immune cells and tumor cells after thaw; evaluation of immunotherapy efficacy on thawed samples	Protocol to assess the antitumor efficacy of an immunotherapeutic peptide in syngeneic orthotopic glioma mouse models	36861832
Martínez-Sabadell et al. 2022	PDX tumor pieces/cells (breast, pancreatic, colorectal, gastric cancers) – frozen after each mouse passage (and optionally pre-implant)	Multiple solid cancers (PDX models)	Re-implantation into mice to continue PDX propagation (preserving an immunotherapy-resistant tumor model); comparative in vivo trials and multi-omics on thawed resistant tumors	Protocol to generate a patient derived xenograft model of acquired resistance to immunotherapy in humanized mice	36317178

Summary

Across multiple studies, Bambanker™ has been successfully used to cryopreserve both solid tumor tissues and single-cell suspensions derived from cancers including melanoma, breast, gastric, colorectal, prostate, pancreatic, and glioblastoma. Researchers utilized Bambanker™ to freeze tumor pieces, cancer-associated fibroblasts (CAFs), dissociated tumor digests, organoids, and genetically modified cell lines, often without requiring a programmable freezer.

Post-thaw, these cells and tissues were used for a variety of functional assays such as 2D and 3D cultures, flow cytometry, scRNA-seq, senescence modeling, enzyme activity assays, and in vivo xenograft implantation. Importantly, studies reported high post-thaw viability, minimal loss in gene expression fidelity, and consistent functional responses compared to fresh samples. This includes successful engraftment of patient-derived xenografts and recovery of immune and stromal populations for microenvironment analysis.

Conclusion

Bambanker™ cryopreservation medium offers a robust, user-friendly solution for preserving cancer cells and tissues across a range of experimental workflows. Its proven performance in maintaining cellular viability, transcriptomic integrity, and functional responsiveness post-thaw makes it an ideal choice for biobanking, downstream molecular analysis, and preclinical modeling. The collective evidence from the literature supports Bambanker's utility in both basic cancer research and translational applications, enabling reliable sample preservation without the complexity of traditional cryopreservation protocols.

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