ATELERIX
SAVE YOUR CELLS

3D MODELS
white paper

Preservation of
organoids & spheroids !!!

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1.0 INTRODUCTION

This report provides data to support the storage and transport of organoids using Atelerix's preservation technologies, STORganoid™ and WellReady™. Organoid models are increasingly used in disease research, drug discovery, and screening applications due to their physiological relevance and ability to better recapitulate in vivo biology. However, their structural complexity and cellular heterogeneity also make them particularly sensitive to handling, storage, and transport conditions.

Organoids are often generated in specialised laboratories and then transferred to separate sites for downstream applications such as imaging, molecular analysis, or high-throughput screening. This separation creates logistical challenges, as delays or suboptimal transport conditions can negatively impact cell viability, structural integrity, and assay performance. Prolonged handling or environmental stress can lead to loss of sensitive cell populations, disruption of 3D architecture, and altered biological responses, ultimately affecting data quality and reproducibility.

While cryopreservation is commonly used to extend the storage window of biological samples, not all organoid types are amenable to freezing. Some organoids are particularly sensitive to freeze-thaw processes, resulting in reduced viability, altered functionality, or complete loss of structural integrity upon recovery. Alternative approaches, such as chemical fixation, are also unsuitable for many applications as they compromise cell viability and prevent functional or longitudinal assays.

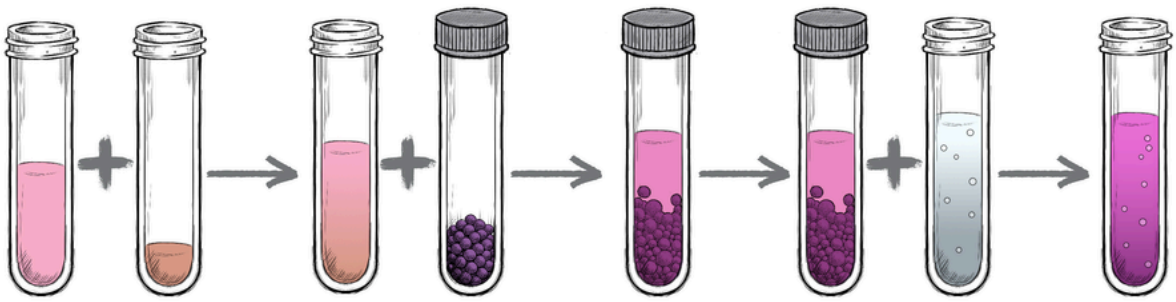
Atelerix's STORganoid™ and WellReady™ technologies provide a solution by enabling ambient storage and transport of organoids and complex models while maintaining viability and functionality, without the need for cold-chain logistics or harsh preservation methods. These technologies are designed to preserve sample integrity and support downstream applications directly.

Importantly, customers are now integrating STORganoid™ and WellReady™ into their workflows to ship organoids in an assay-ready format for downstream use, including screening applications. This enables greater flexibility in experimental design, supports batch processing, and improves consistency and scalability, ultimately enhancing the efficiency and reliability of organoid-based research.

2.0 METHODS

2.1 STORGANOID™

STORganoid™ is designed for the in-vial encapsulation of organoids or spheroids in suspension with medium.



1. Add Organoid/ Spheroid suspension to vial of Gel A

2. Add Organoids/ Spheroids & Gel A to GelBase Beads

3. Store or Ship

4. Add Dissolution Buffer to Release

5. Retrieve Organoids/ Spheroids

2.2 WELLREADY™

WellReady™ is designed for the in-plate encapsulation of organoids or spheroids in a plate format.

1. Gel A and Gel B

2. Gelation Buffers

3. Wash

4. Gel C & Store

ATELERIX STORGANOID™

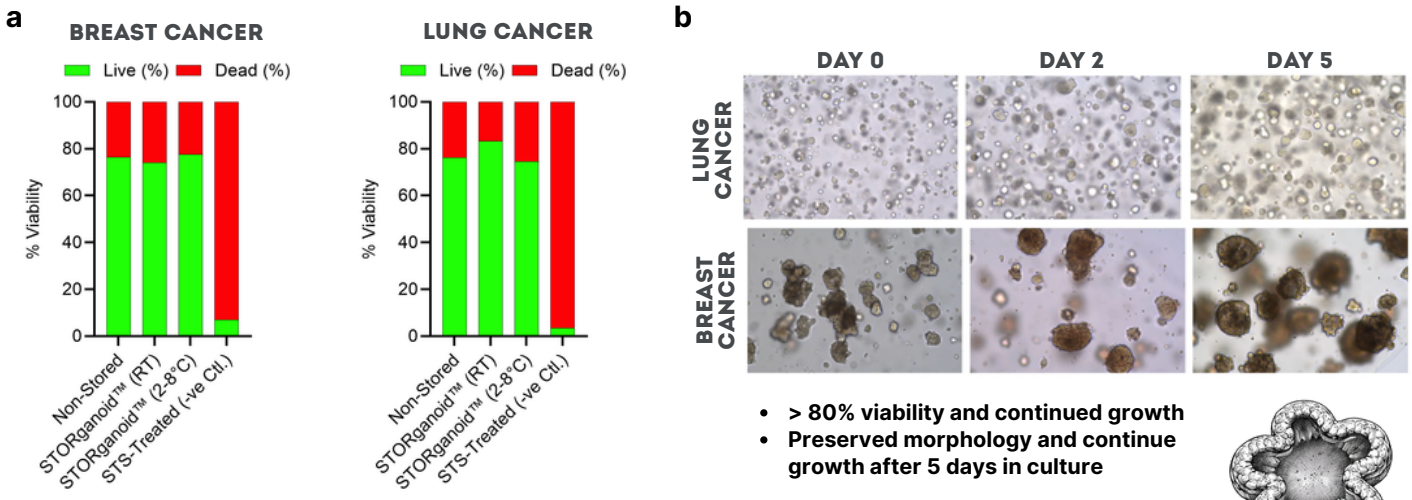
Preservation of Cancer Organoids using STORganoid™ and WellReady™

The use of organoids for disease modelling, drug discovery and personalised medicine has become increasingly popular. Processes for the generation and maintenance of organoids can be complex, requiring high technical awareness. Additionally, some organoid models are unsuitable for storage and shipment by freeze/thaw. STORganoid™ offers a simple solution to these challenges, allowing for the shipment of developed organoid models at ambient temperatures, supplying the end-user with a ready to use model.

OVERVIEW

Cancer organoids are powerful models for oncology research and drug discovery, as they recapitulate tumour heterogeneity and patient-relevant responses. This study demonstrates the effective preservation of cancer organoids using STORganoid™. Breast and lung cancer organoids were resuspended in medium and encapsulated using STORganoid™-M at densities of up to 625,000 organoids per vial. Following storage for one week at either room temperature or 2–8 °C, the organoids were released and assessed for viability. Results showed no loss in viability after 7 days of storage, and organoids resumed normal culture with continued healthy growth for up to 5 days post-release, maintaining their functional integrity.

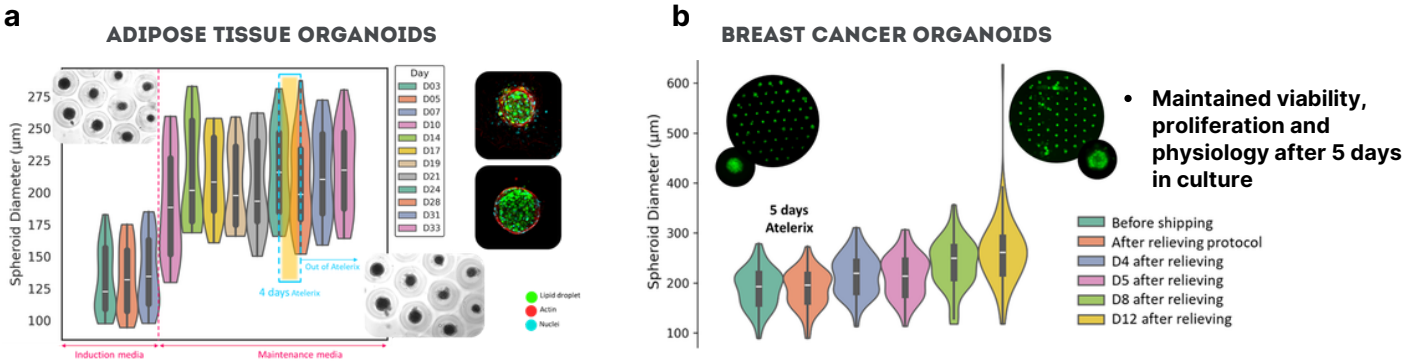
Preservation of cancer organoids.



- **> 80% viability and continued growth**
- **Preserved morphology and continue growth after 5 days in culture**

Preservation of cancer organoids using STORganoid™. Breast and lung cancer organoids were resuspended in medium before being encapsulated using STORganoid™-M at densities up to 625,000 organoids/vial. After storage for 1 week at either room temperature or 2-8°C, organoids were released and assessed for % viability (a) before returning to normal culture conditions for up to 5 days (b).

Preservation of adipose & triple-negative breast cancer organoids.



- **Maintained viability, proliferation and physiology after 5 days in culture**

Preservation of adipose and Triple-Negative-Breast-Cancer (TNBC) organoids using WellReady™. (a) Adipose-derived stem cells (ADSCs) were cultured for 33 days to generate self-assembled organoids. A total of 4,000 cells were seeded per microwell in SmartSphero plates (55 µwells per well, 24-well format). Adipogenic differentiation was induced over the first 7 days, during which cells aggregated and differentiated into pre-adipocytes and mature adipocytes. From day 7 onward, cultures were maintained in maintenance media, promoting organoid growth, as indicated by increasing diameter. At day 24 (~225 µm), organoids were embedded in Atelerix hydrogel for 4 days at room temperature. Following release at day 28, a slight decrease in diameter was observed; however, organoids remained viable and continued to grow over the subsequent 5 days. Endpoint imaging confirmed the presence of mature, unilocular adipocytes (green), indicating preserved adipocyte physiology without hydrogel-induced dedifferentiation. (b) Triple-negative breast cancer MDA-MB-231 cells were cultured for 15 days to form self-assembled spheroids using SmartSphero plates (5,000 cells per spheroid; 55 microwells per well in a 24-well format). Spheroids formed within 3 days, reaching ~200 µm in diameter, and were subsequently embedded in Atelerix hydrogel for 5 days at room temperature. Following release, no reduction in spheroid diameter was observed, indicating no hydrogel-induced cell loss. Spheroids remained viable and proliferative, as demonstrated by continued growth until day 12. Live/dead staining at endpoint showed predominantly viable cells (green) with minimal cell death (red). Data generated by Cherry Biotech.

ATELERIX WELLREADY™

Storage of liver organoids at room temperature using WellReady™

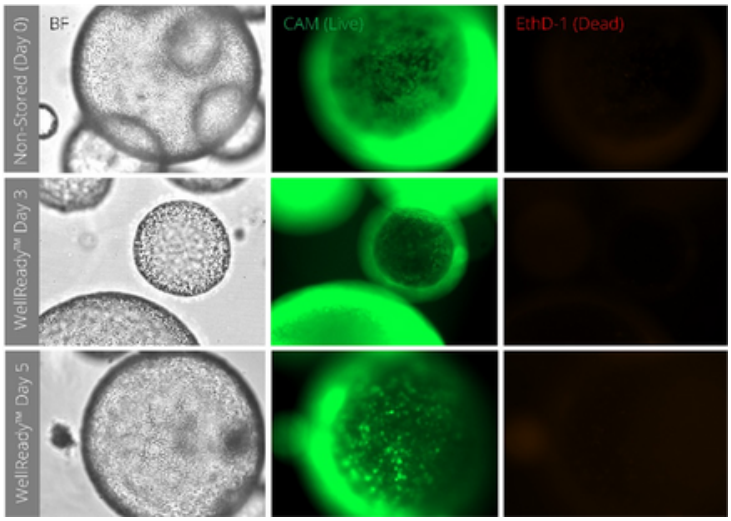
Despite significant advancements in organoid platforms, their generation and long-term culture require significant technical expertise and complex workflows. WellReady™ addresses these challenges by providing fully developed, assay-ready in vitro platforms that eliminate the need for specialist culture procedures at the end-user site. By enabling ambient temperature shipment, WellReady™ delivers ready-to-use organoid models directly to the user, simplifying workflows and accelerating experimental timelines.

OVERVIEW

Liver organoids are important in drug discovery and toxicology due to their drug metabolising capability. This study found that liver organoids can be stored for 5 days at room temperature in ready-to-ship plates using WellReady™, maintaining their morphology, viability and function, and structural integrity. iPSC-derived murine liver organoids were cultured in a Matrigel matrix in 48 well plates. Once mature, the organoids were stored at 20°C for 5 days using WellReady™. Upon release, the organoids retained:

- **High viability and continued growth**
- **Structure and hepatic marker expression**
- **Functionally active drug metabolising enzymes after 72 hours in culture.**

a



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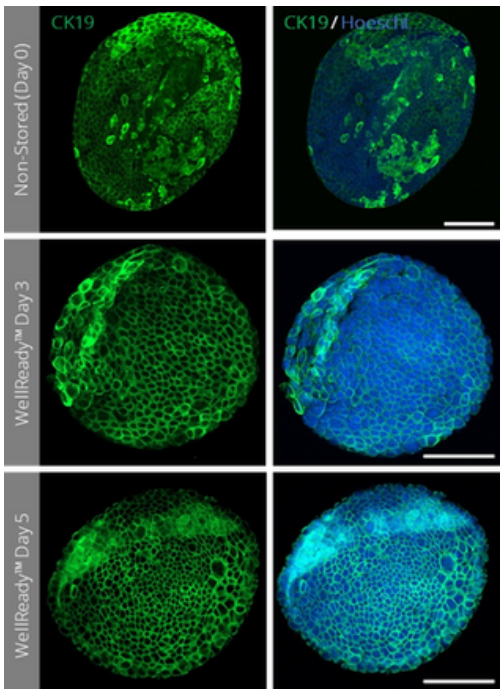

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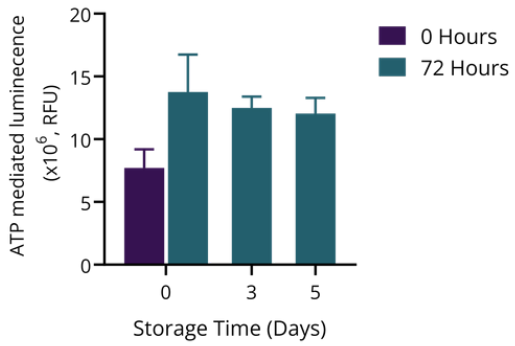

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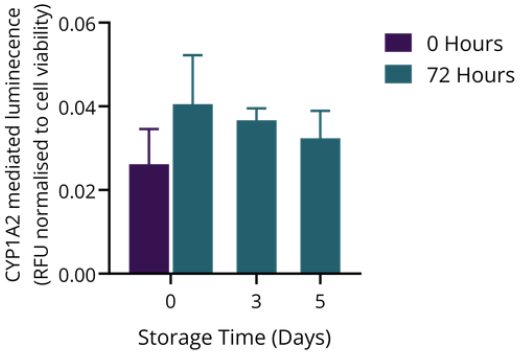

The effect of WellReady™on Liver organoid preservation.

Liver organoids were preserved at 20°C for 5 days using WellReady™. Following preservation, the organoids were released from WellReady™ and returned to culture for 72 hours. before carrying out assays. (a) Organoids were returned to culture overnight and stained with calcein-AM (CAM, green) and ethidium homdimer-1 (EthD-1, red) dyes to visualise live and dead cells respectively. (b) Organoids were returned to culture for overnight, fixed, and stained for the hepatocyte cholangiocyte marker - Cytokeratin-19 (CK19, green) and Hoechst nuclear stain (blue). (c) Cell viability assessed by measuring ATP levels using the CellTitre-Glo® assay, (d) Functional activity assessed by measuring the activity of Cytochrome P450 1A2 using the P450-Glo™ CYP1A2 Assay. Legend indicates post release culture periods. Scale bars represents 100µm.

Atelerix was founded in 2017 with the mission of improving the storage and shipment of biological samples at room temperature, enabling the life sciences sector access to quality biological material.

Superior samples and materials equal better results!

WELLREADY™

4.0 CONCLUSION

Improving the preservation and transport of organoid models opens valuable opportunities across drug discovery, toxicology, and personalised medicine. Maintaining organoids in a viable and functional state is critical to ensure reliable experimental outcomes and reproducible data. A key challenge for biopharma and research organisations lies in the storage and shipment of complex 3D organoid systems, particularly given their sensitivity to environmental changes and the limitations of conventional preservation methods such as freeze–thaw, which can compromise structure and function. In addition, logistical constraints, including transport times and the need for ready-to-use models upon arrival, further complicate their use in distributed research settings.

This report highlights Atelerix's STORganoid™ and WellReady™ technologies, developed to address these challenges by enabling ambient preservation and transport of organoids. STORganoid™ demonstrates successful preservation of breast and lung cancer organoids, for up to 7 days at room temperature or 2–8°C. Post-release, organoids maintain high viability, continue to proliferate, and retain normal physiology. WellReady™ supports the shipment of in-plate, assay-ready organoids, demonstrating successful storage of complex cancer and adipose organoids including triple-negative breast cancer models and adipose organoids for up to 7 days at room temperature or 2–8°C, and liver organoids at room temperature for up to 5 days in ready-to-ship formats. Following preservation, organoids retain structural integrity, high viability, continue to proliferate, express key hepatic markers, and demonstrate active drug-metabolising enzymes after return to culture.

By simplifying logistics and preserving biological integrity, these technologies provide a robust solution for delivering high-quality, ready-to-use organoid models, ultimately supporting more efficient and reliable research outcomes.

"Oooooh, I need to get myself some!"

6.0 ACKNOWLEDGEMENTS

Atelerix gratefully acknowledges the contributions of Cherry Biotech to the work presented in this whitepaper. The experimental studies were conducted by their team, drawing on their recognised expertise in organoid biology and access to high-quality, physiologically relevant models. Atelerix provided the preservation technologies evaluated, while Cherry Biotech were responsible for study execution, data generation, and analysis. Their collaborative engagement and willingness to share data and insights have been critical in assessing and demonstrating the performance of the technologies described. Atelerix sincerely appreciates their scientific rigour, technical excellence, and valued partnership.