

# Encapsulation of live tumor fragments in Elephas Live™ Protect preserves the native tumor microenvironment during ex vivo culture

## Purpose

This white paper presents data demonstrating that Elephas Live™ Protect, a proprietary hydrogel and element of the Elephas Live™ Platform, preserves features of the native tumor microenvironment (TME) in live tumor fragments (LTFs) during ex vivo culture.

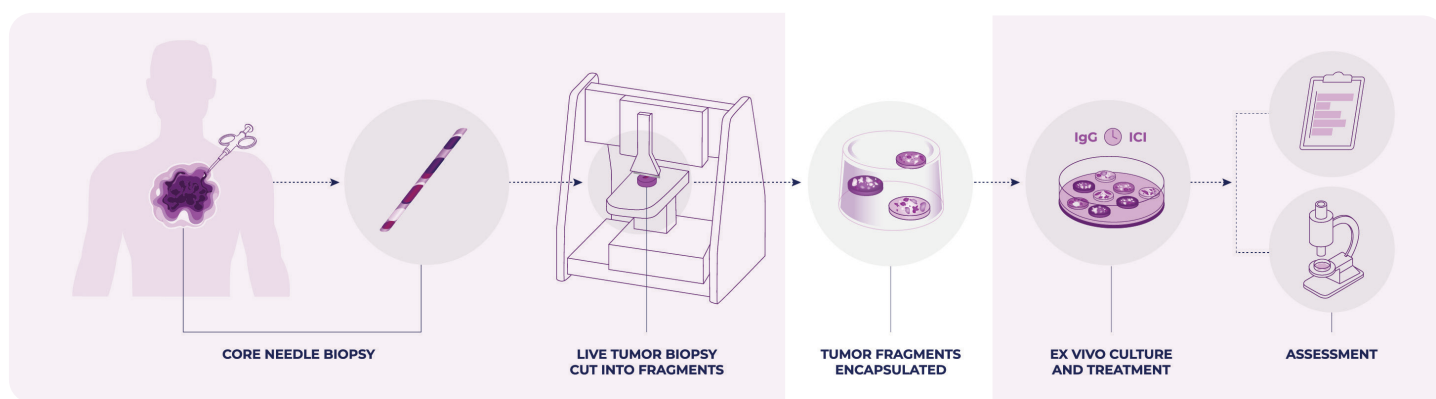
## Introduction

### Problem Statement

The TME is a complex mixture of immune cells, stromal cells, tumor cells, and extracellular matrix that plays a critical role in tumor progression and patient response to immunotherapy<sup>1-7</sup>. Therefore, maintenance of the native TME and retention of infiltrating lymphocytes in ex vivo profiling of tumor tissue offers the greatest potential for accurately predicting patient response to immunotherapy. Elephas Live™ Protect is a proprietary hydrogel that preserves the native TME in LTFs created from core needle biopsies (CNBs) and maintains cell viability over 72 hours of ex vivo culture, enabling cytokine profiling to predict potential response to immunotherapy on the Elephas Live™ Platform.

### The Elephas Live™ Platform enables ex vivo profiling of live tumor tissue

The Elephas Live™ Platform enables ex vivo culture of LTFs created from CNBs to assess response to immunotherapy<sup>8</sup>. Core needle biopsies are cut into LTFs approximately 300µm in thickness using Elephas Live™ Edge, an automated cutting instrument (Figure 1). LTFs are then encapsulated in Elephas Live™ Protect and treated using a sequential treatment strategy (Elephas Live™ Method) in which IgG control followed by ICI are added to the same well. Cytokine profiling at defined time points is used to assess response to immunotherapy.



**Figure 1.** An overview of the Elephas Live™ Platform. LTFs from human core needle biopsies are cut with Elephas Live™ Edge, encapsulated in Elephas Live™ Protect, sequentially treated with IgG control followed by ICI, and assessed for response by cytokine profiling.

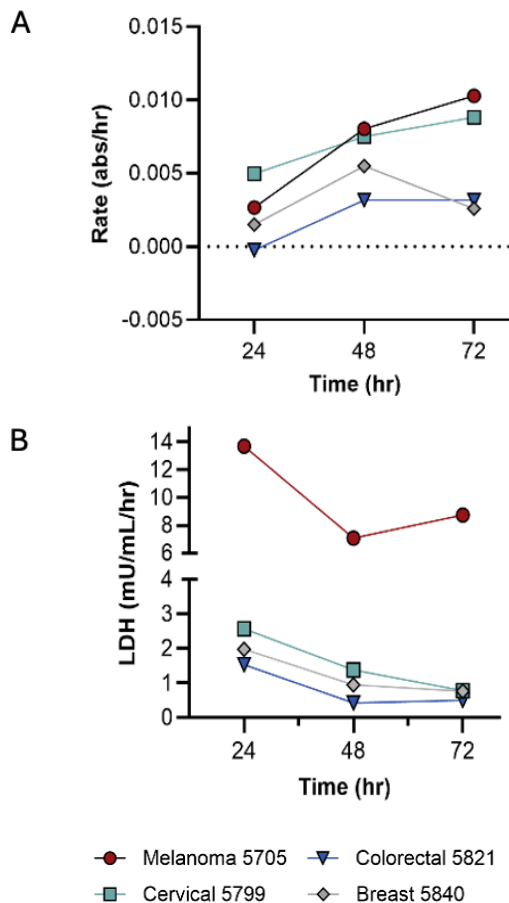


## Results

### Encapsulation in Elephas Live™ Protect preserves LTF cell viability through 72 hours of ex vivo culture

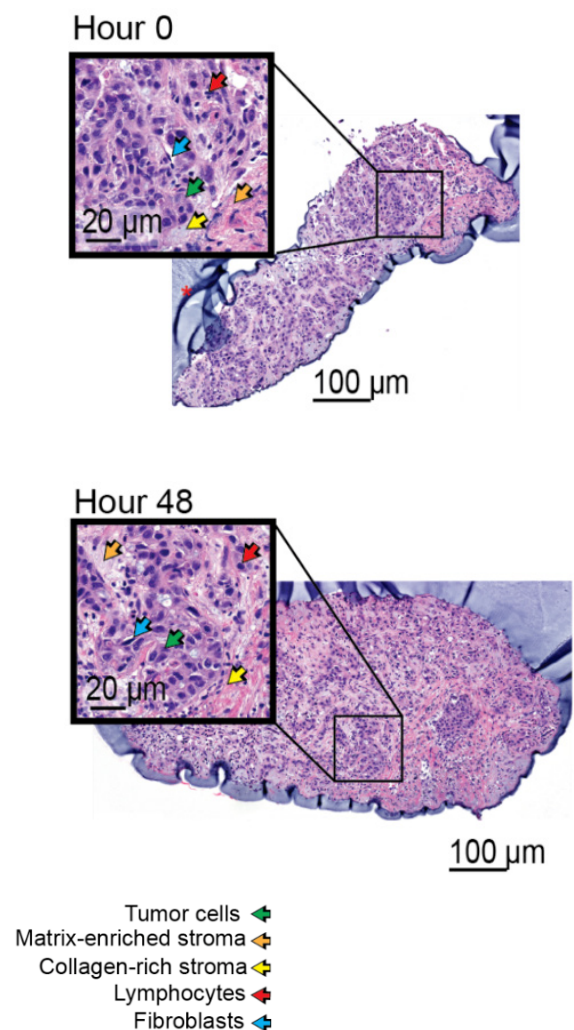
LTFs from 4 different human tumor specimens were encapsulated in Elephas Live™ Protect and cultured through 72 hours. Cell viability increased slightly over time for 3 out of 4 specimens (Figure 2A), while cell death decreased from 24 to 48 hours, then stabilized (Figure 2B). These results highlight that cell health of LTFs is maintained over 72 hours in culture.

LTFs encapsulated in Elephas Live™ Protect retain histological features through 48 hours in culture.



**Figure 2.** Cell health of encapsulated LTFs over 72 hours in culture. A) Changes in cell viability of LTFs from 4 different human tumor specimens assessed by CCK8 assay, B) Changes in cell death as measured by LDH release from cells in LTFs from the same 4 tumor specimens.

Representative images of LTFs encapsulated in Elephas Live™ Protect at hour 0 and at hour 48 of ex vivo culture show tumor cells, lymphocytes, fibroblasts, and stroma are identifiable at the start of culture and after 48 hours, underscoring that Elephas Live™ Protect preserves histological features (Figure 3).



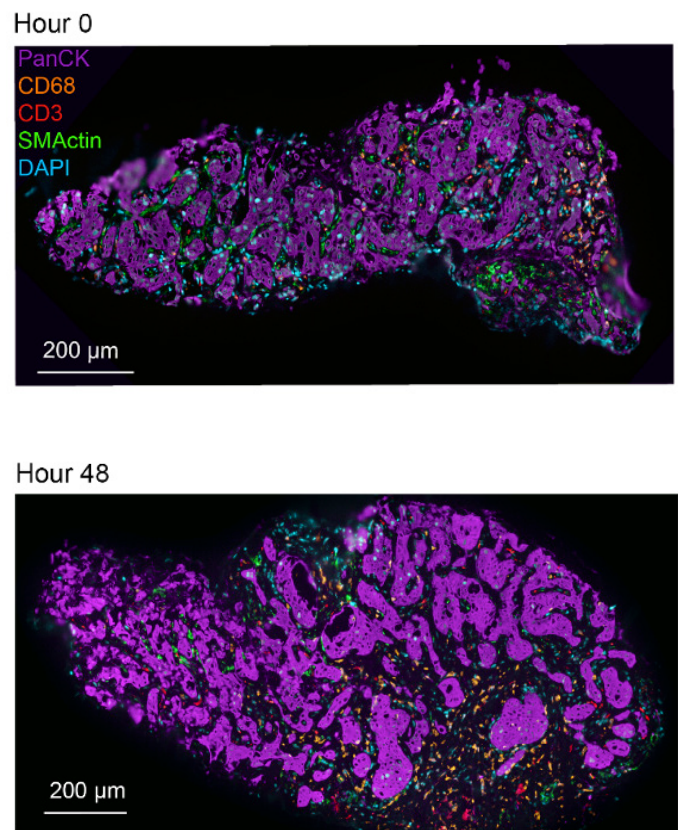
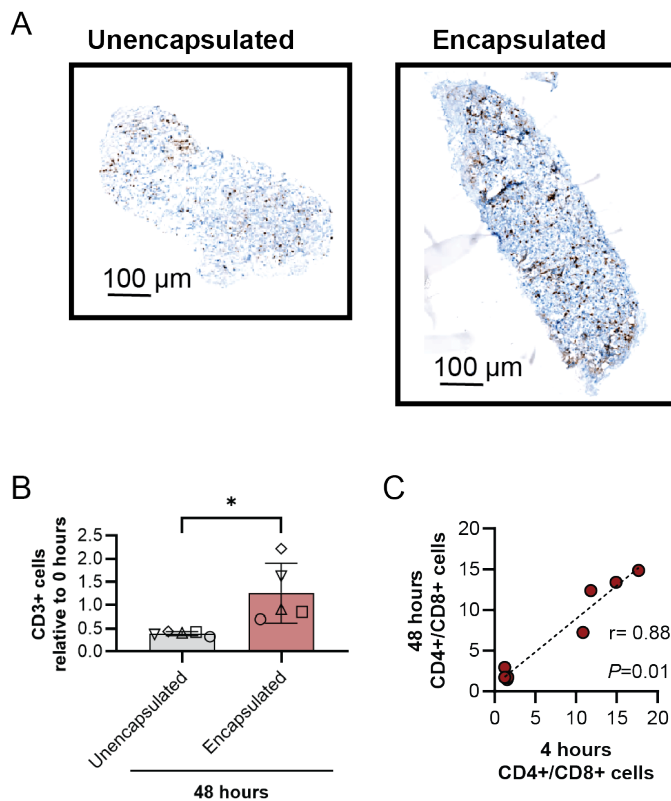
**Figure 3.** H&E-stained sections of LTFs from human liver cancer encapsulated in Elephas Live™ Protect at 0 hours and 48 hours. At both time points, tumor cells (green arrows), lymphocytes (red arrows), fibroblasts (blue arrows), and stroma (matrix-enriched stroma, orange arrows; collagen-enriched stroma, yellow arrows) are identifiable.



## Encapsulation in Elephas Live™ Protect preserves features of the native TME

Immunohistochemistry of unencapsulated compared to encapsulated LTFs showed more frequent CD3+ staining in the Elephas Live™ Protect encapsulated LTF (Figure 4A). Quantification of CD3+ cells in unencapsulated and encapsulated LTFs showed that encapsulated LTFs retain significantly more T cells over time than unencapsulated LTFs (Figure 4B). Further, comparison of the percentages of CD4+ and CD8+ cells by IHC at 4 and 48 hours revealed that the ratio of these cell types was maintained over time (Figure 4C).

Further, multiplex immunofluorescence of LTFs encapsulated in Elephas Live™ Protect showed the presence of tumor cells, macrophages, T cells, and stroma, and that these cell types were preserved after 48 hours in culture (Figure 5). Taken together, these data highlight that Elephas Live™ Protect retains features of the native TME over 48 hours.



**Figure 4.** T cells are better retained in encapsulated LTFs. A) IHC staining of CD3+ cells in unencapsulated and encapsulated humanized PDX LTFs after 48 hours in culture, B) Quantification of CD3+ cells at 48 hours relative to 0 hours for unencapsulated vs. encapsulated LTFs, where each symbol represents 1 tumor (N=5 tumors per group, \* indicates  $P < .05$ ), C) Comparison of percentages of CD4+ and CD8+ cells at 4 and 48 hours ( $n = 9$  human tumors;  $r = 0.88$ ,  $P = 0.01$ ).

**Figure 5.** Multiplex immunofluorescence labelling of encapsulated human liver LTFs at 0 hours and 48 hours in culture showed the presence of tumor cells (PanCK – magenta), macrophages (CD68 – orange), T cells (CD3 – red), and stroma (SMAActin – green).



## Conclusion

Elephas Live™ Protect is a proprietary hydrogel that encapsulates live tumor fragments and allows for the diffusion of nutrients and metabolites, which supports cell viability throughout ex vivo culture. The hydrogel is permeable to various treatments, including antibodies and small molecules, facilitating functional profiling of tumor tissue on the Elephas Live™ Platform. Importantly, Elephas Live™ Protect maintains the cellular composition of the native TME and prevents T-cell egress in LTFs over 48 hours in culture, which is essential for determining response to immunotherapy in tissue specimens analyzed on the Elephas Live™ Platform.

Contact Elephas to learn more about how the **Elephas Live™ Platform** uses live tumor tissue to predict response to immunotherapy.

## Methods

### LTF Generation

CNBs of 12- to 20-gauge were cut into LTFs using an automated, proprietary cutting instrument. The LTF slices were cut at a 20° angle, with a thickness of 300 µm. LTFs were counted manually and equally distributed into the wells of a 24-well plate.

### Encapsulation

After LTFs were plated, 300 µL of Elephas Live™ Protect was added to each well. LTFs were exposed to a 395-nm UV light for 90 seconds to polymerize the hydrogel and washed three times with DPBS. After polymerization, 500 µL of culture media was added to each well for downstream analysis. Specimens were maintained at 37°C and 5% CO<sub>2</sub> throughout culture.

### Assays

#### Viability and cytotoxicity assays:

Relative viability was assessed using Cell Counting Kit-8 (CCK8) (Abcam, ab228554). The CCK8 reagent was added immediately after plating, and media was sampled approximately 24 hours later to measure absorbance at 450 nm. Media and reagent were refreshed daily, and the rate of absorbance over time (abs/hour) was calculated. Relative cytotoxicity was measured by the LDH-Glo™ Cytotoxicity Assay kit (Promega, J2381) per manufacturer's instructions.

#### Histology:

Unencapsulated LTFs were fixed directly in 10% phosphate buffered formalin (Fisher Scientific SF100-4), while

encapsulated LTFs remained in Elephas Live™ Protect for fixation. Formalin fixed LTFs were paraffin-embedded and sectioned at 5-µm thickness on a rotary microtome (Leica, RM2255) and stained with hematoxylin and eosin (H&E) or processed for immunohistochemistry (IHC) or multiplex immunofluorescence (mIF) using a Ventana Medical Systems Discovery Ultra Autostainer. Primary antibodies (against CD3, CD68, and pan-cytokeratin) and secondary antibodies were from Ventana Medical Systems. The smooth muscle actin antibody was from Cell Marque. DAB chromogenic detection kit and FAM, Rhodamine, and Cy5 fluorophore detection kits were from Ventana Medical Systems. Opal 780 was from Akoya Biosciences.

## References

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