User Manual

elive™ Gel

For encapsulation of live tumor fragments ex vivo to maintain native tumor microenvironment for up to 72 hours as part of the elive™ platform

elive™

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The elive™ Platform

The elive™ Platform enables ex vivo profiling in settings of limited tissue

The elive™ platform enables ex vivo culture of live tumor fragments (LTFs) created from core needle biopsies (CNBs) to assess response to immunotherapy. CNBs are cut into LTFs approximately 300 µm in thickness

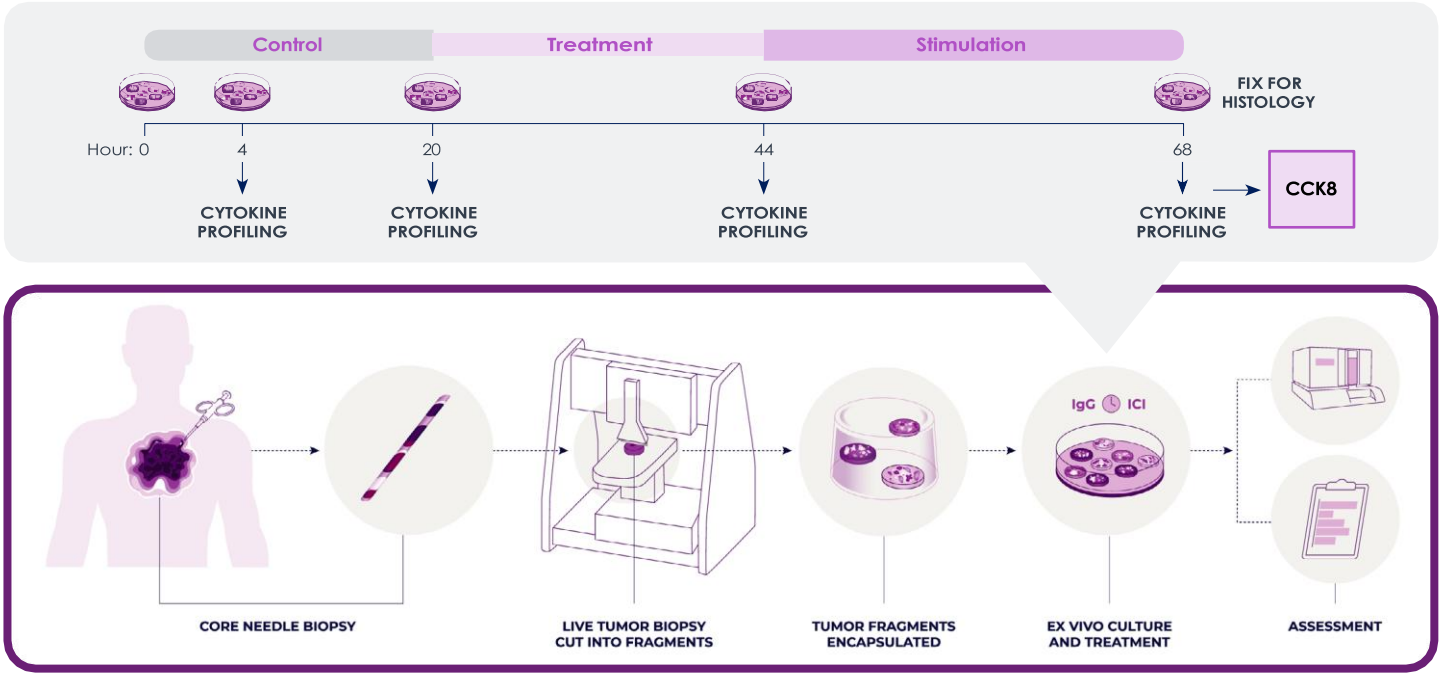


Figure 1. An overview of the elive™ platform, highlighting elive™ Method. LTFs are created by cutting human CNBs on elive™ Edge. LTFs are encapsulated in elive™ Gel and treated using elive™ Method, an approach that applies IgG control (0-20 hours) and ICI (20-44 hours) sequentially to the same tissue in a single well. Response to ICI is assessed by cytokine profiling at the indicated time points.

The elive™ platform comprises elive™ Edge, an automated cutting instrument that creates LTFs from CNBs, and elive™ Gel, a proprietary hydrogel that preserves features of the native tumor microenvironment (TME).

Additionally, the platform incorporates elive™ Method, a novel sequential treatment strategy that addresses the challenge of intraspecimen heterogeneity in a setting of limited tissue.

Unlike conventional approaches that rely on formalin-fixed paraffin-embedded (FFPE) tissue, the elive™ platform profiles live tumor tissue from CNBs, without freezing, allowing a rapid assessment of treatment response. The platform preserves large contiguous areas of TME, which improves the ability to accurately predict response to immunotherapy.

Cytokine profiling is performed longitudinally to assess treatment response. Together, these components enable a novel form of immunotherapy response assessment—live tumor profiling that preserves the native TME and captures functional cytokine responses to treatment.

Related Resources

- elive™ Edge User Manual
- elive™ Method User Manual
- Human Tissue Culture Media User Guide

Product Description

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 response to immunotherapy. Maintenance of the native TME and retention of infiltrating lymphocytes in ex vivo pro-filing of tumor tissue offers the greatest potential for accurately predicting response to immunotherapy.

elive™ Gel 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 elive™ platform.

Encapsulation of LTFs in elive™ Gel helps to maintain the native microenvironment and histological features by limiting cell egress while still allowing for the diffusion of nutrients. The hydrogel is also permeable to various treatments, including antibodies and small molecules.



Warnings and Precautions

- This product is for research use only.
- Biosafety: Handle human tissue in accordance with institutional biosafety protocols. Human tissue samples may contain bloodborne pathogens or other transmissible agents. Always follow universal precautions and approved handling procedures and personal protective equipment to minimize exposure risk.
- Chemical and media spills: Exercise caution when working with buffers and tissue media. Clean up any spills or splashes immediately according to established safety procedures and notify appropriate laboratory personnel.
- Fixation fumes: Fixation chemicals may emit hazardous fumes; perform fixation steps in a fume hood whenever possible.
- Safety Data Sheets: Review and understand the Safety Data Sheet (SDS) for each hazardous chemical prior to use.
- Sharps safety: Handle scalpels, forceps, and other sharp tools with caution.
- Perform all work outlined in these instructions in a biosafety cabinet (BSC), except UV exposure for lights designated for use outside the BSC in Table 1.
- UVA light emitters have a very high intensity at short distances. Do not look at UVA light.
- The product should not be used past the expiration date on the tube.
- The product is stable for up to 4 freeze-thaw cycles.
- Store the product in an ultracold freezer (-60 to -90°C). Product should not be outside ultracold storage for more than 30 minutes.

Required Materials and Equipment

Materials

- Tissue culture media (if using within elive™ platform, contact Elephas for the Human Tissue Culture Media User Guide)
- DPBS (Gibco 14190-144, or equivalent)
- Ice buckets
- Metal forceps
- Tap water
- Thermometer
- Wet ice
- 24-well culture plate (Falcon, 353047)

Equipment

- Appropriately sized pipettes
- Appropriately sized pipette tips
- Biosafety cabinet (BSC)
- Incubator, set to 37°C and 5% CO2
- Benchtop microcentrifuge
- Timer
- Ultracold freezer (-60 to -90°C)
- UV light and accessories (recommendations and specifications listed in Table 1)

Table 1. Recommended UV Lights and Specifications

Manufacturer	Waveform Lighting	UViTRON International	DYMAX
UV Light	Waveform RealUV™ (MFG PN 7022.95)	SkyRay 800 (MFG PN UV3153)	BlueWave® AX-550 V2.0 System with PrimeCure® (MFG PN 60878, 60879, or 60880)
Wavelength	395 nm	385 nm	385 nm
Accessories	Elephas Light Stand (PN 100040) Available from Elephas upon request	Flood Stand & Shield Kit (MFG PN UV4393)	BlueWave® AX-550 Stand with Acrylic Back-Shield (MFG PN 43410) Three-Sided Acrylic Shield (MFG PN 41395)
Height*	2.625 +/- 0.125 in.	8 in.	Top of stand: ~9.6 in.
Intensity/Power Setting	N/A	10%	10%
Plate Position Under UV Light	Centered	Centered	Centered
Exposure	90 sec.	90 sec.	90 sec.
Plate Lid	Off (BSC)	On (bench)	On (bench)
Chamber	N/A	Not recommended	Not recommended

*from the bottom of the UV light to the bottom of the plate

Instructions

Preparation

- Set up the UV light according to the specifications listed for your light in the Recommended UV Lights and Specifications table.
- Obtain a bucket of cool water (between 18 – 22°C), and a second bucket of wet ice.

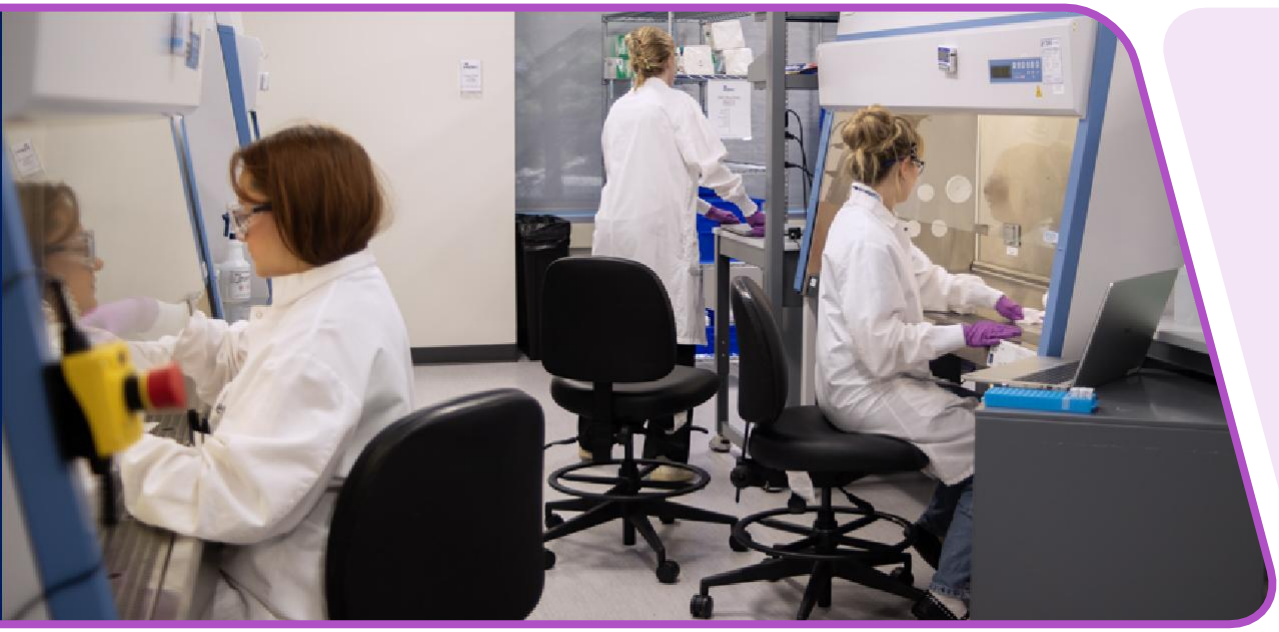
Instructions

1. In a BSC, add 100 µL of culture media to the wells to be encapsulated.
2. Transfer tissue fragments into prepared wells using a metal forceps.
    a. If tissue fragments are bloody complete polymerization may not occur. Follow the washing steps in the troubleshooting section.
    b. Handle tissue gently to minimize mechanical damage.
3. Thaw elive™ Gel in a bucket with cool tap water for 5 minutes ensuring tube is floating and not fully submerged.
    a. One tube of elive™ Gel contains 700 µL and is sufficient for two culture wells.
    b. Start timer at the beginning of the thaw. elive™ Gel has a time limit of 30 minutes outside of the -80°C freezer.
4. Once thawed rapidly invert tube to mix and spin in benchtop centrifuge for ~5 seconds. Place elive™ Gel in wet ice until use. Only the cap of the tube should be above the ice level.
5. Remove tissue culture media from the well(s), taking care not to disturb the tissue.
6. Turn off light in BSC. Mix elive™ Gel by pipetting up and down. Transfer 300 µL to each well taking care not to create bubbles during pipetting.
7. Ensure fragments are evenly dispersed in well prior to polymerization by gently swirling the plate. If necessary, gently adjust fragments with metal forceps or spatula.
    a. Take care to minimize fragment overlap as much as possible.
    b. Ensure no bubbles are present in hydrogel. If bubbles appear, carefully pop using a pipette tip.
8. Center the plate under the UV light, with the lid on or off as listed in Table 1for your light, and expose for exactly 90 seconds.
9. Add 1000 µL of DPBS to the well to rinse. Wait 1 minute, then remove buffer. Repeat the rinse process twice more (total of three rinses).
10. Return hydrogel to the ultracold freezer (-60 to -90°C). Product is stable up to 4 freeze-thaw cycles.
11. Add 500 µL of tissue culture media with appropriate treatment to each well containing tissue.
12. Place plate in the incubator at 37°C and 5% CO2.

Troubleshooting

If tissue is bloody, perform the following additional steps during washing:

1. Carefully trim away any visible blood clots, while preserving the paler tissue regions, if feasible.
2. Fill two 60 x 15 mm non-treated petri dishes with 3 mL of culture media.
3. Using non-serrated forceps, transfer all the tissue pieces into the petri dish.
4. Gently shake the petri dish three times in both north–south and east–west directions, followed by three circular swirls to wash the tissue pieces.
5. Transfer the washed tissue pieces into the second petri dish pre-filled with media.
6. Repeat the shaking and swirling steps to continue washing.
7. Once most of the visible blood is removed, carefully transfer the washed tissue pieces using non-serrated forceps into the designated well(s) of a culture plate.



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