User Manual

elive™ Method

For ex vivo assessment of response to an immune checkpoint inhibitor in live tumor fragments 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.

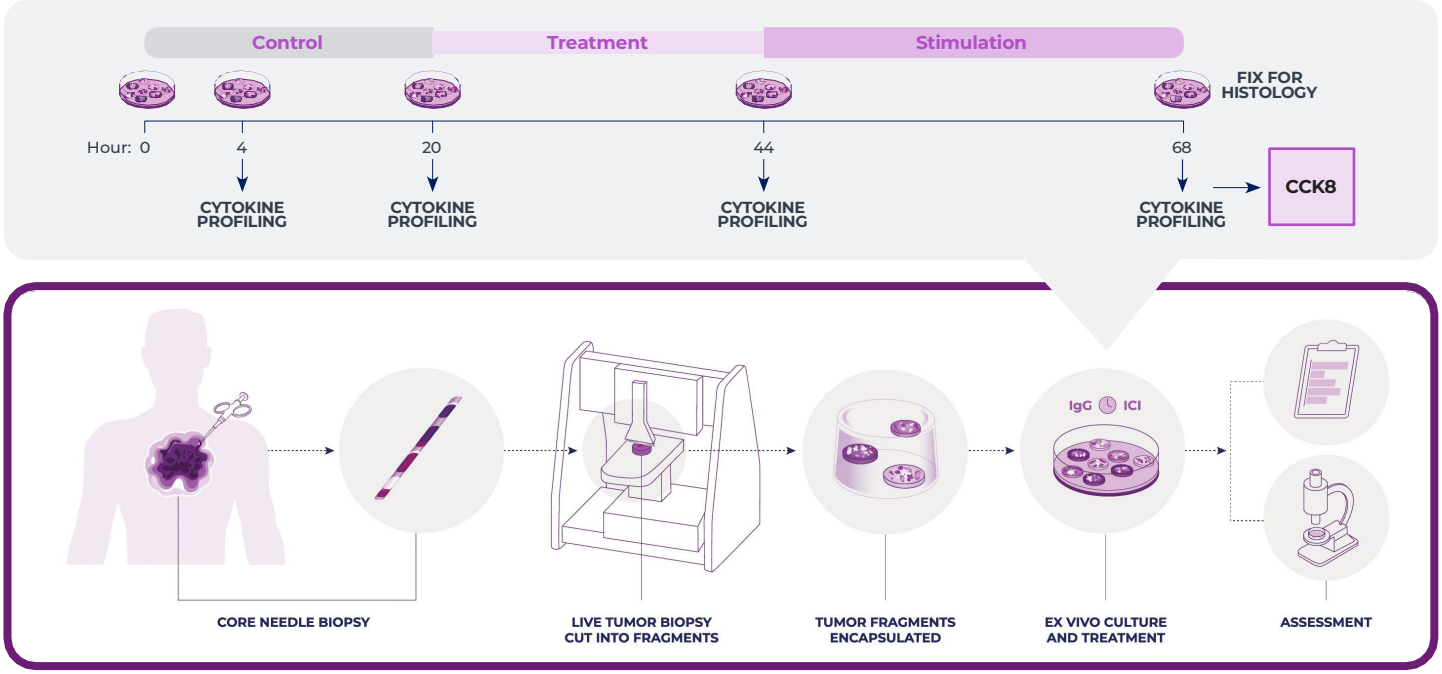


Figure 1. An overview of the elive platform, highlighting elive Method. LTFs are created by cutting human CNBs on elive Edge. LTFs are encapsulated in elive Gel and treated using elive Method, an approach that applies IgG control (0-20 hours) and ICI (20-44 hours) sequentially to the same tissue in a 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.

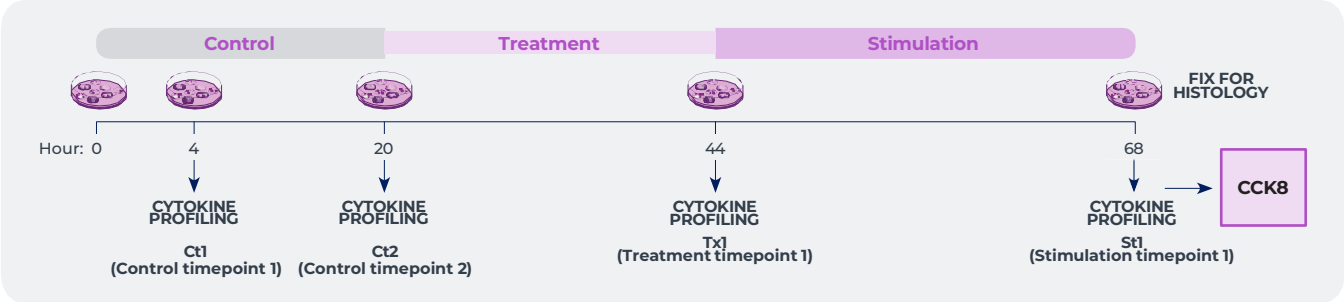
Related Resources

- elive Edge User Manual
- elive Gel User Manual
- Human Tissue Culture Media User Guide

Product Description

The elive Method is an ex vivo workflow within the elive platform designed to assess immune checkpoint inhibitor (ICI) response in LTFs.

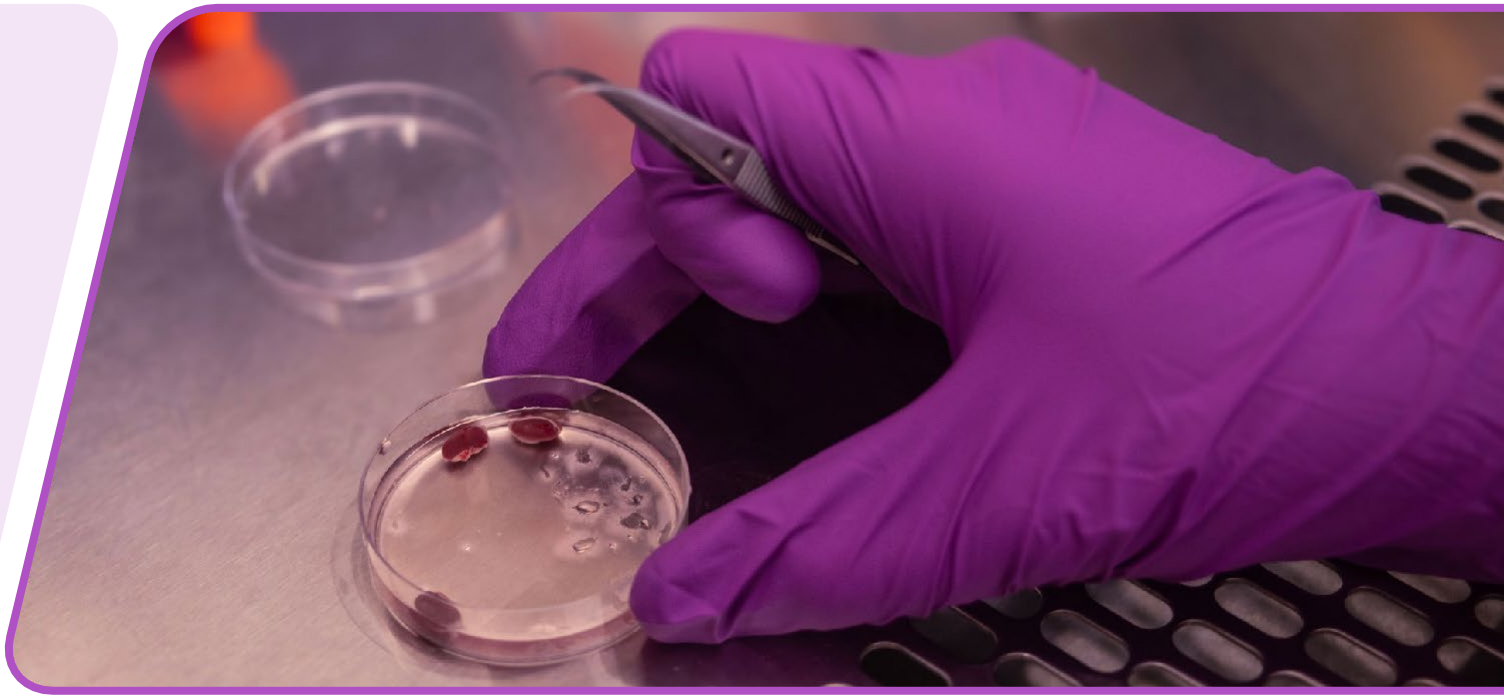
1. LTFs obtained from standard-of-care CNBs are encapsulated to preserve the native TME and cultured through sequential control and treatment phases in a single well.
2. During the control phase, LTFs are treated with IgG isotype control antibody, and culture media is collected for cytokine profiling.
3. The treatment phase then begins in the same well with ICI, test therapeutic, or combination, followed by additional media collection.
4. Following the treatment phase, a anti-CD3/anti-CD28 stimulation phase is introduced to evaluate T-cell functional capacity within the same LTFs, followed by a final media collection timepoint. Cytokines in the media are profiled using a multiplex Luminex assay to determine cytokine production rates for each of the control, treatment, and stimulation phases.
5. Terminal tissue viability is assessed using the Cell Counting Kit 8 (CCK8), and samples are fixed for histopathological evaluation. This method provides a robust and scalable approach for assessing functional immune responses in ex vivo tumor models, supporting translational research and therapeutic development.



A schematic of the three phases of elive Method through 68 hours of ex vivo culture.

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 (PPE) 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.



Acronyms and Definitions

Term	Definition
αCD3/αCD28	Anti-cluster of differentiation 3/anti-cluster of Differentiation 28
αCTLA-4	Anti-cytotoxic T-lymphocyte-associated antigen 4
αLAG3	Anti-lymphocyte activation gene-3
αPD-1	Anti-programmed cell death protein 1
αPDL-1	Anti-programmed cell death ligand 1
CCK8	Cell Counting Kit 8
CNB	Core needle biopsy
Ct1	Control timepoint 1
Ct2	Control timepoint 2
Tx1	Treatment timepoint 1
St1	Stimulation timepoint 1
IgG	Immunoglobulin G
LTF	Live tumor fragment

Required Materials and Equipment

Materials

- Agarose, low melt (LM)
- 24 well tissue culture treated plate
- elive Gel
- Human tissue culture media, refer to User Guide for more information
- DPBS (Gibco, 14190-144)
- Cell Counting Kit 8 (WST-8/CCK) (Abcam, AB228554-101)
- 96-well, polystyrene, flat-bottom
- 10% phosphate buffered formalin
- Alcohol, reagent ACS grade, 70%, 95% and 100%
- Xylene, histology grade
- Hematoxylin Stain
- Eosin Y solution
- Hydrochloric acid, 37% ACS reagent
- IgG isotype control
- Desired treatment
- ImmunoCult Human αCD3/αCD28 T Cell Activator (StemCell Technologies, 10971)
  Multiplex human cytokine panels:
  - Luminex Human XL Cytokine Fixed 30-plex Panel Kit (R&D Systems, FCSTM18B-30)
  - Human Discovery Assay Custom 17-plex Panel (R&D Systems, CUSTOM-LXSA-H-17)

Equipment

- elive Edge
- Biosafety cabinet (BSC)
- Incubator (37°C, 5% CO_2)
- Refrigerator (2–5°C)
- Ultracold freezer (−60 to 90°C)
- Luminex LX200 Instrument

Instructions

Tissue processing

- For CNBs, cut the sample using the elive Edge instrument to generate LTFs.
- For forceps biopsies, manually dissect the tissue into pieces using a scalpel or surgical scissors, carefully trim away visible blood clots while preserving paler regions, and wash the cut pieces in media twice with appropriate precautions before plating.

LTF Plating

1. After cutting the specimen, count the total number of LTFs obtained and determine the appropriate number of wells to plate in a 24-well culture plate. As biopsies can vary in size, the number of wells will depend on the available tissue. Refer to Table 1 for guidance on minimum and maximum LTF counts for plating a single well and scale accordingly based on the total LTF count.
2. For forceps biopsy pieces, follow the plating instructions designated for 18-gauge CNBs.
3. Dispense 100 µL of culture media into the wells, gently transfer the LTFs, and ensure they remain fully submerged.

CNB Gauge Size	Min. LTFs per well	Max. LTFs per well
20 gauge	5	30
18 gauge	5	15
16 gauge	2	8
14 gauge	2	4
12 gauge	2	2

Table 1

Treatment Preparation

4. Prepare treatments and controls based on the total number of culture wells plated.
5. Prepare the IgG control at 50 µg/mL (1× concentration) in tissue culture media, using 600 µL per well with a 10% overage and ensuring the appropriate IgG isotype control is used.

$$\frac{50ug/mL x\ (0.600\,mL\ x\ \#\ of\ wells\ x\ 1.1)}{starting\ concentration\ of\ stock\ IgG\frac{ug}{mL}} = \ Volume\ of\ stock\ IgG\ needed\ (mL)$$

6. Prepare the treatment at the desired final concentration in tissue culture media. Refer to the appendix for recommended treatment concentrations and vendor information.

Day 0 – Encapsulation with elive Gel & Control Media Addition

7. Encapsulate LTFs according to elive Gel instructions.
8. Add elive Gel to one blank well (without LTFs). This will serve as the reference well for the CCK8 absorbance read.
9. Add 500 µL of 50 µg/mL control (IgG) media to each well containing LTFs and 500 µL of fresh tissue culture media to the blank well.

Day 0 – Control Supernatant Pull (Ct1)

10. At 5 ± 1 hour post-control (IgG) treatment addition collect 100 µL of culture media per LTF containing well into Ct1 tube(s) and record timestamp. Store tube(s) in ultracold (-60 to -90°C) freezer.
11. Replenish each LTF containing well with 100 µL of control (IgG) media.

Day 1 – Control Supernatant Pull (Ct2) & Treatment Addition

12. At 22 ± 4 hours post-control (IgG) treatment addition collect 100 µL of culture media per LTF containing well into Ct2 tube(s) and record timestamp. Store tube(s) in ultracold (-60 to -90°C) freezer.
13. Replenish each LTF containing well with 100 µL of test therapeutic.

Day 2 – Treatment Supernatant Pull (Tx1)

14. Prepare the anti-CD3/anti-CD28 stimulation treatment for all wells according to manufacturer's working concentrations, ensuring sufficient volume for all treated wells plus one additional CCK8 blank well, and include a 10% overage to account for pipetting loss.

Wells*	CCK8 Reagent (µL)	Human Tissue Culture Media (µL)	aCD3/aCD28 (µL)
1	55	41.25	13.75
2	110	82.5	27.5

Example guidelines. *Includes 10% overage.

15. At 46 ± 4 hours post-control (IgG) treatment addition, collect 100 µL of culture media per LTF containing well into Tx1 tube(s) and record timestamp. Store tube(s) in ultracold (-60 to -90°C) freezer. Discard 100 µL from the blank well.
16. Replenish all wells (including blank) with 100 µL stimulation media.

Day 3 – CCK8 Plate Read and Stimulation Supernatant Pull (St1)

17. At 70 ± 4 hours post-control (IgG) treatment addition, transfer 100 µL culture media from all wells including the blank well to a clean flat bottom 96-well plate.

18. Using a plate reader and corresponding software measure absorbance at 450 nm for all wells.
19. After reading, collect 100 µL of culture media per LTF containing well into St1 tube(s) and record timestamp. Store tube(s) in ultracold (-60 to -90°C) freezer.
20. Using the Protocol Data Upload module in Elephas portal, upload measured absorbance values from CCK8 assay.

Day 3 – Fixation for Histology

21. Remove any residual supernatant and add 2 mL of 10% formalin to each LTF-containing well.
22. Allow the tissue to be fixed for a minimum of 12 hours, then use forceps to transfer the formalin-fixed encapsulated tissue samples to 70% alcohol.
23. Proceed with standard histology processing as desired.

Multiplex Cytokine Assay

24. Thaw the collected culture media supernatants at 4°C until no ice crystals remain (approx. 30 minutes). Avoid repeated freeze–thaw cycles. A maximum of three freeze–thaw cycles is recommended.
25. Add 25 µL of sample directly to the appropriate wells of the Luminex assay plate and mix with the recommended diluents according to the Luminex cytokine assay kit instructions.
    a. To account for limited supernatant volume, samples may be plated as single replicates. It is recommended that assay standards are run in duplicate.
26. Perform the Luminex 30-plex and/or 17-plex cytokine assays on all collected culture media time-point aliquots. Refer to R&D Systems kit instructions and the Luminex instrument manual for more information.
27. Launch the Cytokines module in Elephas portal and create a new plate. Using the Plate List, upload the Luminex CSV files generated from the cytokine assay. Analyzed data will be available in CSV format and delivered based on the terms of account agreement following analysis by Elephas.

Best Practices

- Handle and move LTFs with non-serrated forceps as serrated forceps may cause mechanical damage.
- When collecting supernatants avoid disturbing the tissue or dislodging the encapsulated puck from the well.
- Do not puncture the gel puck instead, gently tilt the plate to guide supernatant toward the edge for easier collection.

Troubleshooting

My LTFs look uneven in size. Is this acceptable?

Yes, minor variation is expected, but significant size disparity may affect assay performance. Confirm the tissue was positioned correctly during cutting. Distribute LTFs evenly among wells if multiple conditions are being tested.

I don't appear to have sufficient number of LTFs to meet the minimum recommendation. What can I do?

If the LTF count is below the recommended minimum for the corresponding CNB gauge size, do not proceed with the assay.

I have more than the maximum number of recommended fragments. Can I run additional wells?

Yes, if additional fragments are obtained after cutting, you may run extra wells on the platform according to your desired experimental conditions.

My fragments dissociate during transfer. How do I keep them intact during plating?

The recommended transfer method is to use non-serrated forceps and move fragments individually to the culture plate. Ensure that LTFs remain hydrated at all times and minimize the amount of pressure applied during handling. Once encapsulated, elive Gel will help maintain tissue integrity.

The LTFs float or move during plating. How do I stabilize them?

Ensure minimal residual liquid before adding elive Gel.

My fragments appear dry during sorting. Does this impact viability?

LTFs should never dry out. Always keep them submerged or intermittently moistened with media to maintain viability prior to plating and encapsulation.

My treatment/supernatant volumes don't match expected well levels. Why?

Confirm:

1. Inclusion of 10% overage
2. Evaporation in the incubator
3. Pipette calibration
4. Proper 5× stock concentration
5. Encapsulant absorbing the media

Timepoint supernatants vary greatly in color. Should I be concerned?

Color changes in the media often reflect changes in pH that can be attributed to metabolic activity, but drastic differences may indicate contamination or media evaporation; practice aseptic processing and verify volumes.

Appendix

Treatment Recommendation

- Refer to the list below for recommended antibodies for treatment media:
  - InVivoSIM anti-human PD-1 (Pembrolizumab biosimilar; BioXCell SIM0010)
  - InVivoSIM anti-human PD-L1 (Atezolizumab biosimilar; BioXCell SIM0009)
  - InVivoSIM anti-human CTLA-4 (Ipilimumab biosimilar; BioXCell SIM0004)
  - InVivoSIM anti-human LAG-3 (Relatlimab biosimilar; BioXCell SIM0015)
- Treatment Preparation - Prepare treatments at a target 5× concentration of 250 µg/mL, using 100 µL per well, and include a 10% overage.

$$\frac{250ug/mL x\ (0.100\,mL\ x\ \#\ of\ wells\ x\ 1.1)}{starting\ concentration\ of\ stock\ treatment\ \frac{ug}{mL}} = Volume\ of\ stock\ stock\ treatment\ needed\ \ (mL)$$

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