INSTRUCTIONS FOR USE

TISSUESPEC® MULTI-ORGAN METASTASIS dECM HYDROGEL KIT

CONTENTS AND STORAGE

Each TissueSpec® Multi-Organ Metastasis dECM Hydrogel Kit contains bone, liver, and lung dECM components and is sufficient to prepare 2 × 0.5 mL of hydrogel per tissue type at a working concentration of 6 mg/mL. Kits are shipped on ice with a natural insulating material. Upon receipt, store all components at 4°C. Do not freeze. **For research use only. Not for human or animal therapeutic or diagnostic use.**

STORAGE TEMPERATURE: 4°C (do not freeze)

KIT CONTENTS:

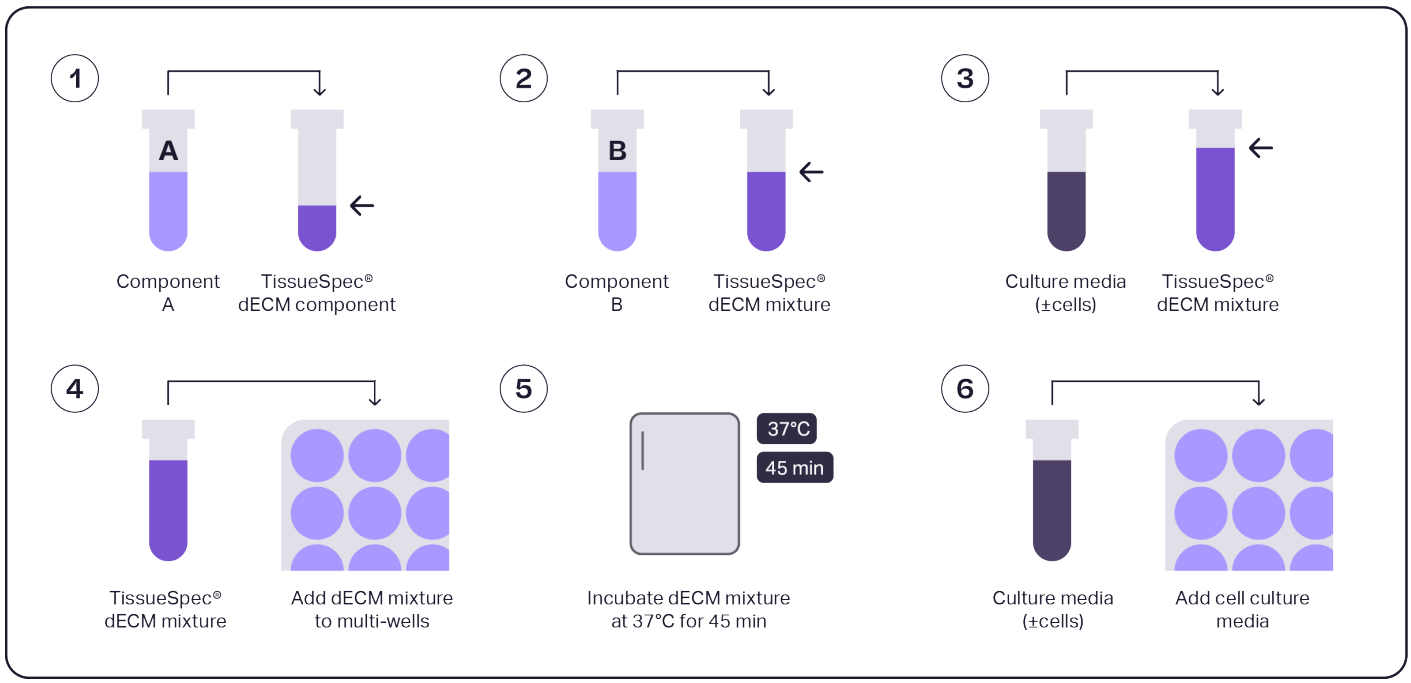
2 x 0.3 mL	TissueSpec® Bone dECM (10 mg/mL)
2 x 0.3 mL	TissueSpec® Liver dECM (10 mg/mL)
2 x 0.3 mL	TissueSpec® Lung dECM (10 mg/mL)
1 x 1 mL	Component A
1 x 1 mL	Component B

PREPARATION OF TISSUESPEC® MULTI-ORGAN METASTASIS dECM HYDROGEL FOR CELL CULTURE

Important: TissueSpec® dECM Hydrogel should be prepared immediately before use and cannot be stored once components are combined.

Important: Please review Instructions for Use prior to proceeding with hydrogel preparation. As hydrogel preparation steps vary depending on whether cells are to be cultured on the surface or encapsulated within hydrogels, please carefully select the appropriate protocol below. Mix thoroughly between each step. Below are instructions to prepare 0.5 mL of TissueSpec® dECM Hydrogel **per tissue type** at a concentration of 6 mg/mL.

Important: To prepare three different TissueSpec® dECM Hydrogels, follow the **same** protocol. The order in which each hydrogel is prepared **can be determined by the user**.



TO CULTURE CELLS ON THE SURFACE OF TISSUESPEC® BONE/LIVER/LUNG dECM HYDROGEL

1. Add 30 µL Component A into the dECM component tube containing 300 µL dECM and mix thoroughly by vortexing.

2. Add 35 µL Component B into the resulting mixture in the dECM component tube and mix thoroughly by vortexing.

3. Add 135 µL cell culture media into the ECM Component tube to yield a final hydrogel concentration of 6 mg/mL. Mix thoroughly by vortexing.

   Note: While we recommend preparation of TissueSpec® dECM Hydrogels at 6 mg/mL, final hydrogel concentration can be adjusted by varying the volume of cell culture media. We recommend spinning the mixture down to remove bubbles.

4. Add hydrogel mixture to the cell culture substrate (e.g., multi-well plate, petri dish) according to your experimental setup. We recommend ~150 µL/cm^2. Please refer to the Appendix for suggested volumes for multi-well formats.

5. Incubate at 37°C in a humidified environment with 5% CO_2 for 45 minutes to achieve gelation.

   Note: A cell suspension at the desired concentration can be prepared at this time.

6. After gelation, gently add cell suspension onto surface of TissueSpec® dECM Hydrogel.

7. Culture cells according to standard cell culture protocols.

   Note: When replacing cell culture media, gently tilt multi-well plate, place pipette tip at edge of the well without touching the hydrogel, and carefully aspirate cell culture media while ensuring hydrogel remains intact at the bottom of the well.

TO CULTURE CELLS ENCAPSULATED WITHIN TISSUESPEC® BONE/LIVER/LUNG dECM HYDROGEL

Note: Harvest or passage cells and prepare 135 µL cell suspension at a known desired cell concentration prior to hydrogel preparation. Optimization may be required.

1. Add 30 µL Component A into the dECM component tube containing 300 µL dECM and mix thoroughly by vortexing.

2. Add 35 µL Component B into the resulting mixture in the dECM component tube and mix thoroughly by vortexing.

   Note: We recommend spinning the mixture down to remove bubbles at this point.

3. Add 135 µL cell suspension into the ECM Component tube to yield a final hydrogel concentration of 6 mg/mL. Mix thoroughly by pipetting up and down.

   Note: While we recommend preparation of TissueSpec® dECM Hydrogels at 6 mg/mL, final hydrogel concentration can be adjusted by varying the volume of cell suspension.

4. Add hydrogel mixture containing cells to the cell culture substrate (e.g., multi-well plate, petri dish) according to your experimental setup. We recommend ~150 µL/cm^2. Please refer to the Appendix for suggested volumes for multi-well formats.

5. Incubate at 37°C in a humidified environment with 5% CO_2 for 45 minutes to achieve gelation and encapsulate cells within hydrogel.

6. After gelation, gently add cell culture media onto TissueSpec® dECM Hydrogel.

> **Note**: When replacing cell culture media, gently tilt multi-well plate, place pipette tip at edge of the well without touching the hydrogel, and carefully aspirate cell culture media while ensuring hydrogel remains intact at the bottom of the well.

RECOMMENDATIONS FOR ANALYSIS

Cells cultured on the surface or encapsulated within TissueSpec® dECM Hydrogel may be assayed, analyzed by microscopy, or fixed and embedded in paraffin and sectioned. Fix cells according to standard formalin or paraformaldehyde fixation protocols.

For gene expression analysis, TissueSpec® dECM Hydrogels can be dissociated with collagenase prior to proceeding with standard RNA isolation protocols.

Please visit **xylyxbio.com/resources/** for detailed Supporting Protocols.

TROUBLESHOOTING TIPS

My TissueSpec® dECM Hydrogel is very viscous and hard to pipette. What can I do?

If the dECM component is difficult to handle, we recommend vortexing the dECM component tube, spinning the tube down to remove bubbles, then leaving the tube at room temperature for 10 minutes before attempting to handle again. For pipetting especially viscous samples, we recommend using larger micropipette tips or cutting off the tip to allow for a larger opening at the end of the micropipette tip.

My TissueSpec® dECM Hydrogel failed to gel. What can I do?

In some cases, improper storage or handling can reduce the ability of the product to form a hydrogel or prolong the incubation time required for gelation. Check the pH of your TissueSpec® dECM Hydrogel preparations prior to adding your cells. pH values should range from 7.0 – 8.0 for gelation. Extending incubation at 37°C to 1 hour or longer may also facilitate gelation.

My cells are not attaching or surviving. What is wrong?

Check the pH of your TissueSpec® dECM Hydrogel preparations prior to adding your cells. pH values should range from 7.0 – 8.0 for cell viability and attachment.

For technical support, please visit **inmatrico.com** or e-mail **info@xylyxbio.com**.

REFERENCES

Duan et al. Hybrid gel composed of native heart ECM and collagen induces cardiac differentiation of human embryonic stem cells without supplemental growth factors. Journal of Cardiovascular Translational Research. 2011.

O'Neill et al. The regulation of growth and metabolism of kidney stem cells with regional specificity using extracellular ECM derived from kidney. Biomaterials. 2013.

APPENDIX

MULTI-WELL PLATE	VOLUME PER WELL
6	1000 – 1500 µL
12	500 – 700 µL
24	300 – 350 µL
48	100 – 150 µL
96	30 – 50 µL