



Instructions for Use

TissueSpec® ECM Hydrogel Kit

Storage temperature -20°C

This kit is sufficient to prepare 2 × 0.5 mL hydrogel at a concentration of 6 mg/mL. For research use only. Not for human or animal therapeutic or diagnostic use.

Contents and Storage

The components of the TissueSpec® ECM Hydrogel Kit are shipped on ice. Upon receipt, store all components at -20°C. Avoid freeze/thaw cycles. Kit components are listed in the table below.

Component	Quantity
TissueSpec® ECM Hydrogel	0.3 mL × 2
A	1 mL
B	1 mL

Preparation of TissueSpec® ECM Hydrogel for Cell Culture

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. Thaw all components to room temperature prior to use. Mix thoroughly by pipetting up and down between each step. Avoid introducing bubbles. Below are instructions to prepare 0.5 mL of TissueSpec® ECM Hydrogel at a concentration of 6 mg/ml.

To culture cells on the surface of TissueSpec® ECM Hydrogel:

1. Add 30 µL Component A into the TissueSpec® ECM Hydrogel component tube containing 300 µL ECM and mix thoroughly by pipetting up and down. Avoid introducing bubbles.
2. Add 35 µL Component B into the TissueSpec® ECM Hydrogel component tube and mix thoroughly by pipetting up and down. Avoid introducing bubbles.
3. Add 135 µL cell culture media into the TissueSpec® ECM Hydrogel component tube to yield a final hydrogel concentration of 6 mg/mL.

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

4. Add hydrogel mixture to the cell culture substrate (e.g., multi-well plate, petri dish) according to your experimental setup. We recommend ~150 $\mu\text{L}/\text{cm}^2$. 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® ECM 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 the bottom edge of the well, and carefully aspirate cell culture media while ensuring hydrogel remains intact at the bottom of the well.

To culture cells encapsulated within TissueSpec® ECM 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 TissueSpec® ECM Hydrogel component tube containing 300 μL ECM and mix thoroughly by pipetting up and down. Avoid introducing bubbles.
2. Add 35 μL Component B into the TissueSpec® ECM Hydrogel component tube and mix thoroughly by pipetting up and down. Avoid introducing bubbles.
3. Add 135 μL cell suspension into the TissueSpec® ECM Hydrogel component tube to yield a final hydrogel concentration of 6 mg/mL .

Note: While we recommend preparation of TissueSpec® ECM 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 $\mu\text{L}/\text{cm}^2$. 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® ECM Hydrogel.

Note: When replacing cell culture media, gently tilt multi-well plate, place pipette tip at the bottom edge of the well, 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® ECM 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, hydrogels can be dissociated with collagenase prior to proceeding with standard RNA isolation protocols. Please visit xylyxbio.com for detailed Supporting Protocols.

Troubleshooting Tips

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

If the TissueSpec® ECM Hydrogel component is difficult to handle, we recommend vortexing the TissueSpec® ECM Hydrogel component tube, spinning the tube down, 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 ECM 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® ECM Hydrogel preparations prior to adding your cells. pH values should range from 7.2 – 7.4 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® ECM Hydrogel preparations prior to adding your cells. pH values should range from 7.2 – 7.4 for cell viability and attachment.

For technical support, please visit xylyxbio.com or email info@xylyxbio.com.

References

1. 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.
2. 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
6	1000 – 1500 µL
12	500 – 700 µL
24	300 – 350 µL
48	100 – 150 µL
96	30– 50 µL