Instructions for Use

TissueSpec® Matrix Hydrogel Kit

Store at -20°C.

This kit is sufficient to prepare 1 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® Matrix 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
Matrix	0.6 mL
A	1 mL
B	1 mL

Preparation of TissueSpec® Matrix 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 4°C prior to use. Mix thoroughly by pipetting up and down between each step. Avoid introducing bubbles. Below are instructions to prepare 1 mL of TissueSpec® Matrix Hydrogel at a concentration of 6 mg/ml.

To culture cells on the surface of matrix hydrogel:

1. Working on ice, add 60 µL Component A to 600 µL Matrix and mix thoroughly by pipetting up and down. Avoid introducing bubbles.

2. Add 70 µL Component B and mix thoroughly by pipetting up and down. Avoid introducing bubbles.

3. Add 270 µL cell culture media to yield a final hydrogel concentration of 6 mg/mL.

   Note: While we recommend preparation of TissueSpec® Matrix 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 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® Matrix 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 matrix hydrogel:

Note: Harvest or passage cells and prepare 270 µL cell suspension at a known desired cell concentration prior to hydrogel preparation. Optimization may be required.

1. Working on ice, add 60 µL Component A to 600 µL Matrix and mix thoroughly by pipetting up and down. Avoid introducing bubbles.

2. Add 70 µL Component B and mix thoroughly by pipetting up and down. Avoid introducing bubbles.

3. Add 270 µL cell suspension to yield a final hydrogel concentration of 6 mg/mL.

   Note: While we recommend preparation of TissueSpec® Matrix 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® Matrix 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® Matrix 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 **eastriverbio.com** for detailed Supporting Protocols.

Troubleshooting Tips

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

Some TissueSpec® matrix products may begin to form a very weak gel inside the tube at temperatures above 4°C, making handling and accurate pipetting difficult. Pease keep your TissueSpec® matrix components refrigerated until immediately prior to use. 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 matrix 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® matrix 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® matrix 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 **eastriverbio.com** or email **info@eastriver.com**

References

1. Duan *et al*. Hybrid gel composed of native heart matrix and collagen induces cardiac differentiation of human embryonic stem cells without supplemental growth factors. *J Cardiovasc Transl Res*. 2011.

2. O'Neill *et al*. The regulation of growth and metabolism of kidney stem cells with regional specificity using extracellular matrix 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

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