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Instructions for Use

TissueSpec™ Matrix Hydrogel Kit

Store at -20°C.

This kit is sufficient to prepare 5 mL hydrogel.
For research use only. Not for use in diagnostic or therapeutic applications.

Contents and Storage

The components of your 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 x 5
A	5 mL
B	5 mL

Preparation of TissueSpec™ Matrix Hydrogel for Cell Culture

Important: Thaw all components at 4°C prior to use. Keep all components cold on ice during hydrogel preparation. Mix thoroughly by pipetting up and down between each step. Do not vortex. Avoid introducing bubbles. Use the instructions below to prepare 1 mL of TissueSpec™ Matrix Hydrogel.

To culture cells on the surface of matrix hydrogel:

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

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

3. Add 270 µL cell culture medium to the Matrix tube and mix thoroughly by pipetting up and down. Avoid introducing bubbles. At this point, the total volume in the Matrix tube should be 1 mL hydrogel mixture. Keep mixture cold on ice.

4. Add an amount of hydrogel mixture to the cell culture substrate (e.g., well plate, petri dish) according to your experimental setup. We recommend ~200 µL/cm^2. See corresponding volumes for multi-well formats below:

Well plate	Volume
6	500 – 600 µL
12	300 – 350 µL
24	250 – 275 µL
48	100 – 125 µL
96	25 – 50 µL

5. Incubate the hydrogel mixture at 37°C in humidified atmosphere with 5% CO_2 for 45 min to achieve gelation.

Tip: You can prepare a cell suspension at your experimental cell concentration during this time.

6. After gelation, gently add cell suspension onto surface of your tissue-specific matrix hydrogel.

7. Culture cells according to your experimental cell culture protocol.

Tip: When replacing cell culture media, gently tilt multi-well plate, place pipette tip at the bottom edge of the well, and carefully aspirate spent 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 cell suspension at a known cell concentration prior to hydrogel preparation. Generally, cells are diluted to between 1 × 10^4 to 1 × 10^5 cells/mL, depending on cell type and experimental conditions. Optimization may be required.

For each 1 mL hydrogel preparation, cells should be re-suspended in a total volume of 270 µL. Use the instructions below to prepare 1 mL of TissueSpec™ Matrix Hydrogel.

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

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

3. Add 270 µL cell suspension (in culture medium) to the Matrix tube and mix thoroughly by pipetting up and down. Avoid introducing bubbles. At this point, the total volume in the Matrix tube should be 1 mL hydrogel mixture. Keep mixture cold on ice.

4. Add an amount of hydrogel mixture to the cell culture substrate (e.g., well plate, petri dish) according to your experimental setup. We recommend ~200 µL/cm^2. See corresponding volumes for multi-well formats below:

Well plate	Volume
6	500 – 600 µL
12	300 – 350 µL
24	250 – 275 µL
48	100 – 125 µL
96	25 – 50 µL

5. Incubate the hydrogel mixture at 37°C for 45 min to achieve gelation and encapsulate the cells within the hydrogel.

6. After gelation, gently add culture medium and culture cells according to your experimental cell culture protocol.

Tip: When replacing cell culture media, gently tilt multi-well plate, place pipette tip at the bottom edge of the well, and carefully aspirate spent 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: treatment with collagenase breaks down the hydrogel, releasing cells and enabling standard RNA isolation protocols.

Troubleshooting Tips

My TissueSpec™ matrix is extremely viscous and hard to pipette. What can I do?

Some TissueSpec™ matrix products will begin to form a weak gel inside the tube at temperatures above 4°C, making handling and accurate pipetting extremely difficult. Pease keep your TissueSpec™ matrix aliquots frozen until you thaw overnight at 4°C prior to use. For pipetting extremely viscous samples, we recommend cooling your pipette tips to 4°C, using larger pipette tips, or cutting off the tip to allow for a larger opening at the end of the pipette tip.

My matrix failed to gel. What can I do?

In some cases, improper storage or handling of matrix can reduce the ability of the matrix to form a gel or prolong the incubation time required for gelation. Be sure to 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?

Be sure to 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@eastriverbio.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.

Rev. Date 13 April 2017