Protocol: TissueSpec® hydrogel dissociation for cell isolation & analysis

This protocol may be used to dissociate TissueSpec® matrix hydrogels for passaging or analysis of cells, organoids, or patient-derived xenografts. For passaging, please refer to **Procedure A**. For RNA extraction, please refer to **Procedure B**.

The difficulty of dissociating TissueSpec® hydrogels may vary and is dependent on the type of TissueSpec® hydrogel, cells, and duration of culture. Optimization may be required for dissociation of TissueSpec® hydrogels in some applications. Please refer to the Troubleshooting section below for additional tips on how to handle hydrogels that may be especially difficult to dissociate.

Materials

- Cell culture media
- Hanks' Balanced Salt Solution (HBSS) with calcium and magnesium, no phenol red (Gibco® 14025092)
- Collagenase Type I (Gibco® 17100017)

Procedure A

Preparation of reagents

1. Prepare a stock solution of collagenase type I by reconstituting collagenase type I powder in HBSS at a concentration of 50 mg/mL, or according to the manufacturer's instructions.
2. Aliquot and store collagenase at –20°C protected from light.
3. Thaw collagenase on ice prior to use. Avoid multiple freeze/thaw cycles.
4. Warm media and HBSS to room temperature prior to use.

Dissociation of TissueSpec® hydrogels

The following procedure is intended for applications in 24-well plates. Reagent volumes for other multi-well formats are provided in **Appendix A**.

1. Culture cells or organoids in TissueSpec® hydrogel according to your cell culture protocol.

At the time of cell/organoid analysis or passaging:

2. Prepare a *working solution* of collagenase by adding 100 µL of 50 mg/mL collagenase per 1 mL cell culture media.
3. Add 300 µL collagenase-media mixture to each well of the 24-well plate containing hydrogel.

   Note: collagenase-media mixture volumes should completely cover the hydrogel. For suggested adjusted volumes for other multi-well formats, please refer to **Appendix A**.
4. Incubate collagenase with hydrogels at 37°C for 30 – 60 minutes, or until hydrogels are fully dissociated. Optimization may be required.
5. Transfer the dissociated contents of wells to tubes for centrifugation.
6. Gently centrifuge cells/organoids. Aspirate the supernatant.

7. Wash cells/organoids to remove any residual hydrogel components or collagenase by adding 1 mL HBSS to each tube, then repeat step 6.

8. Optional: For greater dissociation of organoids, use a syringe to pass organoids through a 20 Gauge needle (diameter: ~600 µm). If necessary, repeat 3 - 4 times.

Your cells/organoids are now ready for re-plating, analysis, or other downstream applications.

Troubleshooting

The dissociation of TissueSpec® hydrogels in some applications may be especially difficult. We recommend the following guidelines for optimizing dissociation of TissueSpec® hydrogels:

- Manual pipetting of hydrogels to facilitate dissociation
- Prolonging the incubation time of collagenase with hydrogels in step 4
- Following gentle centrifugation in step 6, adding fresh collagenase-media mixture and incubating fresh collagenase at 37°C for additional time.

Procedure B

RNA extraction from cells cultured in TissueSpec® hydrogels

1. Remove TissueSpec® hydrogels from wells by pipette, micropipette, spatula, scoopula, scalpel, or other instrument.
2. Transfer TissueSpec® hydrogels into RNase-free tubes.
3. Add 0.5 – 1 mL of TRIzol (or other phenol reagent suitable for RNA extraction) to each tube.
4. Use a tissue homogenizer to obtain clear, homogenous solutions.
5. Vortex each tube for 30 seconds.
6. Incubate samples at room temperature for 5 minutes to completely dissociate nucleoprotein complexes.
7. Continue with RNA extraction protocol according to the manufacturer's instructions.

For technical support, please visit **eastriverbio.com** or email **info@eastriver.com**

Appendix A

Collagenase-media mixture volumes for multi-well formats:

Multi-well plate	Volume
96 well	50 µL
48 well	150 µL
24 well	300 µL
12 well	0.5 mL
6 well	1 mL

Rev. 8 June 2018