Supporting Protocol

TissueSpec® ECM Hydrogel Dissociation for Cell Isolation and Analysis

This protocol may be used to dissociate TissueSpec® ECM Hydrogels for isolation, analysis, or passaging of cells, organoids, or patient-derived xenografts.

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

Materials (required but not provided)

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

Procedure

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® ECM 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® ECM Hydrogel according to your cell culture protocol.

   At the time of 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 TissueSpec® ECM Hydrogel.

   Note: collagenase-media mixture should completely cover the gel. Recommended volumes for other multi-well formats are provided in Appendix A.

4. Incubate collagenase with TissueSpec® ECM Hydrogels at 37˚C for 30 – 60 minutes, or until TissueSpec® ECM Hydrogels are fully dissociated. Optimization may be required.

5. Transfer the dissociated contents of wells to tubes for centrifugation.

6. Gently centrifuge the dissociated contents. Aspirate the supernatant.

7. Wash cells to remove any residual TissueSpec® ECM 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.

   Cells are now ready for analysis or other downstream applications. For isolation of RNA, refer to **Appendix B**.

Troubleshooting

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

- Manual micropipetting of TissueSpec® ECM Hydrogels to facilitate dissociation.
- Prolonging the incubation time of collagenase with TissueSpec® ECM 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.

Appendix A

Recommended volumes of collagenase-media mixture for multi-well formats:

Multi-well plate	Volume
6	1000 µL
12	500 µL
24	300 µL
48	150 µL
96	50 µL

Appendix B

To isolate high-quality RNA, we recommend using the *TissueSpec® ECM Hydrogel Dissociation for Cell Isolation and Analysis* protocol with one of the following procedures, which may be used for isolation of RNA from cells cultured in TissueSpec® ECM Hydrogels:

A. Isolation of RNA using phenol chloroform method
B. Isolation of RNA using QIAGEN RNeasy Mini Kit

Isolation of RNA using phenol chloroform

1. Complete the *TissueSpec® ECM Hydrogel Dissociation for Cell Isolation and Analysis* protocol.
2. Add 0.5 – 1 mL of TRIzol (or other phenol reagent suitable for RNA extraction) to each tube.
3. Homogenize samples using a tissue homogenizer.
4. Vortex samples for 30 seconds.
5. Incubate samples at room temperature for 5 minutes to dissociate nucleoprotein complexes.
6. Proceed with RNA isolation protocol according to the manufacturer's instructions.

Isolation of RNA using QIAGEN RNeasy Mini Kit

1. Complete the *TissueSpec® ECM Hydrogel Dissociation for Cell Isolation and Analysis* protocol.
2. Add 350 – 500 µL of QIAGEN RLT buffer to each tube.
3. Mix samples by pipetting.
4. Optional: Homogenize samples using a tissue homogenizer.
5. Proceed with RNA isolation protocol according to the manufacturer's instructions.