SUPPORTING PROTOCOL

DISSOCIATION OF TISSUESPEC® dECM HYDROGEL FOR CELL ISOLATION AND ANALYSIS

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

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

MATERIALS (required but not provided)

- cell culture media
- Hank's Buffered Salt Solution (HBSS) with calcium and magnesium, no phenol red (Gibco® 14025092)
- Collagenase Type I (Gibco® 17100017)

PROCEDURE

1. Preparation of Reagents

i. 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.

ii. Aliquot and store collagenase at –20°C protected from light.

iii. Thaw collagenase on ice prior to use. Avoid multiple freeze/thaw cycles.

iv. Warm media and HBSS to room temperature prior to use.

2. Dissociation of TissueSpec® dECM Hydrogels

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

Culture cells or organoids in TissueSpec® dECM Hydrogel according your cell culture protocol.

At the time of cell/organoid analysis or passaging:

i. Prepare a working solution of collagenase by adding 100 µL of 50 mg/mL collagenase per 1 mL cell culture media.

ii. Add 300 µL collagenase-media mixture to each well of the 24-well plate containing TissueSpec® dECM Hydrogel.

> **Note**: collagenase-media mixture volumes should completely cover the TissueSpec® dECM Hydrogel. For suggested adjusted volumes for other multi-well formats, please refer to the **Appendix A**.

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

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

v. Gently centrifuge cells/organoids. Aspirate the supernatant.

vi. Wash cells/organoids to remove any residual TissueSpec® dECM Hydrogel components or collagenase by adding 1 mL HBSS to each tube, then repeat the previous step (step v.).

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

- Manual pipetting of TissueSpec® dECM Hydrogels to facilitate dissociation.
- Prolonging the incubation time of collagenase with TissueSpec® dECM Hydrogels in step iii.
- Following gentle centrifugation in step v., adding fresh collagenase-media mixture and incubating fresh collagenase at 37°C for additional time.

APPENDIX A

MULTI-WELL PLATE	TISSUESPEC® dECM HYDROGEL VOLUME PER WELL
6	1500 µL
12	1000 µL
24	500 µL
48	300 µL
96	100 µL

APPENDIX B

To isolate high-quality RNA, we recommend using the TissueSpec® dECM 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® dECM Hydrogels:

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

A. Isolation of RNA using phenol chloroform

1. Complete the TissueSpec® dECM 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.

B. Isolation of RNA using QIAGEN RNeasy Mini Kit

1. Complete the TissueSpec® dECM 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.