

## Data Sheet

**Intestine dECM Gel Hydrogel Kit**

3D CC Hydrogel

**Cat. # CC172****pack size:Kit**FOR RESEARCH USE ONLY  
NOT FOR USE IN DIAGNOSTIC PROCEDURES  
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Store at: 2-8°C

**Background**

The cellular microenvironment directly and indirectly effects cellular behavior via both biophysical and biochemical routes. The microenvironment is composed of extracellular matrix proteins (ECM), other surrounding cells, cytokines, growth factors, hormones, and other bioactive agents and nano/microscale mechanical properties such as physical tension/stiffness. Decellularization is the process used in biomedical engineering to isolate the native extracellular matrix (ECM) of a tissue from its inhabiting cells, leaving an ECM scaffold of the original tissue, which can be used in cell culture and tissue regeneration. Compared to other tumor derived basement membrane extracts (Matrigel®), these dECM hydrogel scaffolds can provide a more physiological environment with increased growth rates for cells without the use of any exogenous growth factors. Our tissue-specific dECM Gel Hydrogel Kits are a series of native decellularized extracellular matrix (ECM) protein solutions derived from a variety of non-diseased porcine organs (bone, heart, liver, kidney, intestine, skin and lung). These hydrogels can be used as traditional 2D ECM coatings or as hydrogels to encapsulate cells for 3D cell culture applications.

**Components**

Intestine ECM Solution (CS226364): 2 ea (0.3 mL)

Component A (CS226431): 1 ea (1 mL)

Component B (CS226430): 1 ea (1 mL)

**Storage and Handling**

Store all components of the dECM Gel Hydrogel Kit at 2-8°C (do not freeze).

**Source**

Porcine Origin

**Quality Control Testing**

pH: 2.0-3.0

Gelation: Forms Gel

Biocompatibility: &gt; 90% Cell Viability

Sterility: No Fungi or Bacteria Detected

Mycoplasma: Negative

PRRS, PRV: Negative

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## Protocols

### Preparation of dECM Hydrogels for Cell Culture

*Note: 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. Mix thoroughly between each step. Below are instructions to prepare 0.5 mL of dECM Hydrogel at a concentration of 6 mg/mL. This kit is sufficient to prepare 2 × 0.5 mL hydrogel at a concentration of 6 mg/mL.*

### Culturing Cells on the Surface of dECM Hydrogels

1. Add 30  $\mu$ L Component A into the ECM Solution containing 300  $\mu$ L ECM and mix thoroughly by vortexing the mixture.
2. Add 35  $\mu$ L Component B into the ECM/Component A mixture from and mix thoroughly by vortexing the mixture.
3. Add 135  $\mu$ L cell culture media into the ECM/Component mixture from step 2 to yield a final hydrogel concentration of 6 mg/mL. Mix thoroughly by vortexing. *Note: While we recommend preparation of dECM Hydrogels at 6 mg/mL, final hydrogel concentration can be adjusted by varying the volume of cell culture media. We recommend spinning the mixture down to remove bubbles.*
4. Add hydrogel mixture to the cell culture substrate (e.g., multi-well plate, petri dish) according to your experimental setup. We recommend  $\sim$ 150  $\mu$ L/cm<sup>2</sup>.
5. Incubate at 37°C in a humidified environment with 5% CO<sub>2</sub> 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 dECM 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.*

### Culturing Cells Encapsulated within dECM Hydrogels

*Note: Harvest or passage cells and prepare 135  $\mu$ L cell suspension at a known desired cell concentration prior to hydrogel preparation. Optimization may be required.*

1. Add 30  $\mu$ L Component A into the ECM Solution containing 300  $\mu$ L ECM and mix thoroughly by vortexing the mixture.
2. Add 35  $\mu$ L Component B into the ECM/Component A mixture and mix thoroughly by vortexing the mixture. *Note: We recommend spinning the mixture down to remove bubbles at this point.*
3. Add 135  $\mu$ L cell suspension into the ECM/Component mixture from step 2 to yield a final hydrogel concentration of 6 mg/mL. Mix thoroughly by pipetting up and down. *Note: While we recommend preparation of dECM 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  $\sim$ 150  $\mu$ L/cm<sup>2</sup>.
5. Incubate at 37°C in a humidified environment with 5% CO<sub>2</sub> for 45 minutes to achieve gelation and encapsulate cells within hydrogel.
6. After gelation, gently add cell culture media onto dECM 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.*

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## Hydrogel Dissociation for Cell Isolation and Analysis

*Note: This protocol may be used to dissociate dECM Hydrogels for analysis or passaging of cells, organoids, or patient-derived xenografts. The difficulty of dissociating dECM Hydrogels may vary and is dependent on the type of dECM Hydrogel, cells, and duration of culture. Optimization may be required for dissociation of dECM Hydrogels in some applications. Please refer to the Troubleshooting section below for additional tips on how to handle dECM Hydrogels that may be especially difficult to dissociate.*

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. Aliquot and store collagenase at  $-20^{\circ}\text{C}$  protected from light. Thaw collagenase on ice prior to use. Avoid multiple freeze/thaw cycles.
2. Prepare a working solution of collagenase by adding 100  $\mu\text{L}$  of 50 mg/mL collagenase per 1 mL cell culture media.
3. Add 300  $\mu\text{L}$  collagenase-media mixture to each well of the 24-well plate containing dECM Hydrogel. *Note: collagenase-media mixture volumes should completely cover the gel.*
4. Incubate collagenase with dECM Hydrogels at  $37^{\circ}\text{C}$  for 30 – 60 minutes, or until dECM 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 dECM Hydrogel components or collagenase by adding 1 mL HBSS to each tube, then repeat step 6.

## Extracting RNA from Cells Cultured in dECM Hydrogels

1. Remove dECM Hydrogels from wells by pipette, micropipette, spatula, scoopula, scalpel, or other instrument.
2. Transfer dECM 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.

## Immunostaining Cells Cultured in dECM Hydrogels

*Note: While dECM Hydrogels are compatible with standard immunostaining techniques, additional steps may be required to optimize staining results. Notably, organized 3D cellular structures can render antigens of interest less accessible to antibodies and thus present technical challenges to immunostaining. Some formalin-fixed paraffin-embedded samples may require the use of antigen retrieval techniques prior to immunostaining. Embedded cells may be distributed across different focal planes and visualization of cells at high magnification without sectioning samples may require confocal microscopy. Autofluorescence of matrix fibers may increase background or otherwise interfere with visualization of cells within dECM Hydrogels.*

### *Immunostaining without dECM Hydrogel Dissociation or Sectioning*

1. Gently aspirate cell culture media while ensuring dECM Hydrogel remains intact at the bottom of the well.
2. Wash dECM Hydrogel samples with PBS. Gently aspirate PBS.
3. Gently add 500  $\mu\text{L}$  4% formaldehyde to fix dECM Hydrogel samples.
4. Incubate at room temperature for 30 minutes. Thicker dECM Hydrogels may require longer fixation times. Optimization may be required.
5. Gently aspirate 4% formaldehyde.
6. Wash dECM Hydrogel samples twice with PBS. Samples are now ready for standard immunostaining protocols.

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### *Immunostaining by Dissociation of Cells from dECM Hydrogels*

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. Aliquot and store collagenase at  $-20^{\circ}\text{C}$  protected from light. Thaw collagenase on ice prior to use. Avoid multiple freeze/thaw cycles.
2. Prepare a working solution of collagenase by adding 100  $\mu\text{L}$  of 50 mg/mL collagenase per 1 mL cell culture media.
3. Add 300  $\mu\text{L}$  collagenase-media mixture to each well of the 24-well plate containing dECM Hydrogel. *Note: collagenase-media mixture volumes should completely cover the dECM Hydrogel.*
4. Incubate collagenase with dECM Hydrogels at  $37^{\circ}\text{C}$  for 30 – 60 minutes, or until dECM 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 to remove any residual dECM Hydrogel components or collagenase by adding 1 mL HBSS to each tube, then repeat step 6.
8. Resuspend cells in culture media and plate onto chamber slides or coverslips.
9. Incubate at  $37^{\circ}\text{C}$  for a few hours (duration may vary with cell type) to allow for cell attachment.
10. Aspirate cell culture media, wash with HBSS, and fix cells with a fixative appropriate for immunostaining.

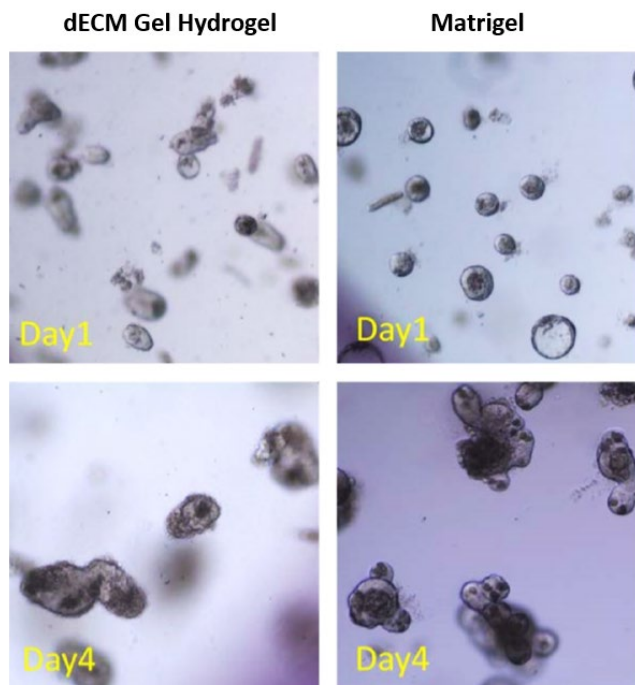
### *Immunostaining of Whole Mount dECM Hydrogels*

1. Gently rinse cells in PBS.
2. Gently add an appropriate volume of 4% formaldehyde to fix dECM Hydrogel samples.
3. Incubate at room temperature for 30 minutes. Thicker dECM Hydrogels may require longer fixation times. Optimization may be required.
4. Wash dECM Hydrogel samples twice with PBS.
5. Dehydrate samples by subsequently incubating samples for 10 minutes across increasing concentrations (v/v) of ethanol in water: 50%, 70%, 95%, 95%, 100%.
6. Transfer samples to 100% CitriSolv (or other clearing agent) for 1 hour at room temperature, followed by 1 hour at  $65^{\circ}\text{C}$ .
7. Remove samples from wells with a razor, scalpel, or other instrument.
8. Transfer samples to a mixture (1:1 by volume) of CitriSolv and micro-cut paraffin for 1 hour at  $65^{\circ}\text{C}$  in a paraffin mold.
9. Transfer samples to 100% micro-cut paraffin for 1 hour at  $65^{\circ}\text{C}$ . *Note: As an alternative to paraffin embedding, samples may be frozen and sectioned and prepared for immunostaining. Following fixation, remove samples from wells with a razor, scalpel, or other instrument and embed in Optimal Cutting Temperature (OCT) compound.*

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## Representative Data



**Figure 1.** *In vitro* culture of mouse intestinal organoids encapsulated in dECM Hydrogels vs. Matrigel. Mouse intestinal organoid crypts exhibit similar *in vivo*-like budding morphologies in dECM Hydrogels compared to Matrigel over a 4 day time period.

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