Sigma-Aldrich®

Data Sheet

Lung dECM Gel Hydrogel Kit

3D CC Hydrogel

Cat. # CC175 **pack size:Kit**

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Store at: 2-8ºC

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

Lung ECM Solution (CS226374): 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: > 90% Cell Viability

Sterility: No Fungi or Bacteria Detected

Mycoplasma: Negative

PRRS, PRV: Negative

EMD Millipore Corporation, 28820 Single Oak Drive, Temecula, CA 92590, USA 1-800-437-7500

Ver 1.0/2020-10-14/CC175-DS/NJA

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 µL Component A into the ECM Solution containing 300 µL ECM and mix thoroughly by vortexing the mixture.
2. Add 35 µL Component B into the ECM/Component A mixture from and mix thoroughly by vortexing the mixture.
3. Add 135 µL 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 ~150 µL/cm^2.
5. Incubate at 37°C in a humidified environment with 5% CO_2 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 µL cell suspension at a known desired cell concentration prior to hydrogel preparation. Optimization may be required.

1. Add 30 µL Component A into the ECM Solution containing 300 µL ECM and mix thoroughly by vortexing the mixture.
2. Add 35 µL 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 µL 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 ~150 µL/cm^2.
5. Incubate at 37°C in a humidified environment with 5% CO_2 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.*

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°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 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 dECM Hydrogel. *Note: collagenase-media mixture volumes should completely cover the gel.*
4. Incubate collagenase with dECM Hydrogels at 37°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.

Extrating 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 µL 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.

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°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 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 dECM Hydrogel. *Note: collagenase-media mixture volumes should completely cover the dECM Hydrogel.*
4. Incubate collagenase with dECM Hydrogels at 37°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°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°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°C in a paraffin mold.
9. Transfer samples to 100% micro-cut paraffin for 1 hour at 65°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.*

Representative Data

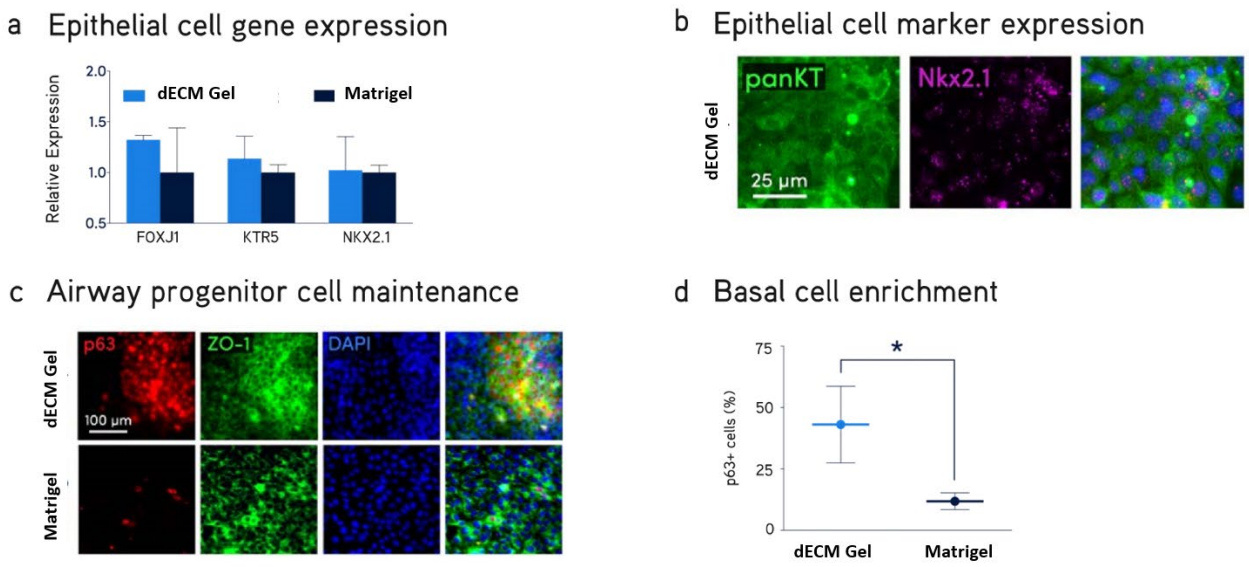

Figure 1. Primary normal human bronchial epithelial (NHBE) cells were cultured on dECM Hydrogels or Matrigel for 10 days. NHBE cells cultured on dECM Hydrogels supported robust expression of normal lung epithelial cell markers FOXJ1, KTR5, NKX2.1, panKT, p63 and ZO-1 (A,B,C) and generated a larger subpopulation of p63+ basal airway cells (D) compared to Matrigel.

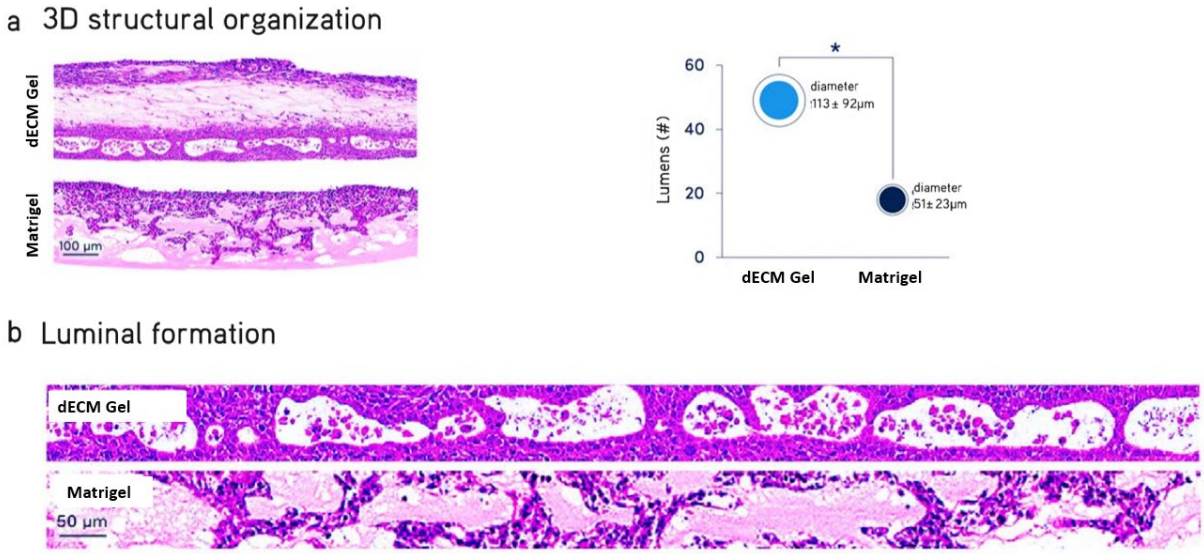

Figure 2. Air liquid interface cultures of primary normal human bronchial epithelial (NHBE) cells after 21 days. NHBE cells cultured in dECM Hydrogels formed more organized and complex stratified luminal structures recapitulating the cellular architecture of the human airway, with significantly larger average diameter compared to Matrigel.