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Catalog Number
CSH-RUO-01

Cultrex™ Synthetic Hydrogel Kit

Product Description

The Cultrex Synthetic Hydrogel Kit provides materials to prepare up to 1 mL of a synthetic, defined hydrogel formulation. Each kit includes 8 ready-to-use components that, when combined, form a robust and effective growth scaffold designed for 3D cell culture models, including organoids. As an improvement to the hydrogel preparation user experience, end users can expect low resistance pipetting of each component to form a pre-gel mixture, and after addition of the final component, the hydrogel can be expected to fully form within 30 minutes.

Intended Use

For Research Use Only. Not for use in diagnostic procedures. The Cultrex Synthetic Hydrogel Kit is designed to support 3D cell culture applications, including organoid expansion and differentiation.

Storage & Handling

For optimal product performance, store kit and components at ≤ -70 °C and protected from light. Use a manual defrost freezer and limit number of freeze-thaw cycles. To best prolong shelf-life, keep component tubes on ice during use unless otherwise specified. Immediately after use, store components at ≤ -70 °C.

Precautions

When handling bio-hazardous materials such as human-derived cells, safe laboratory procedures should be followed, and protective clothing should be worn.

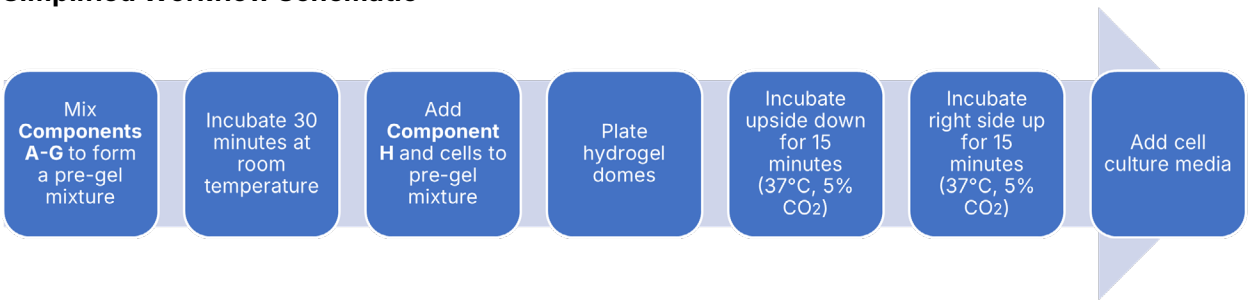
Technical Hints

- This package insert must be read in its entirety before using this product.
- Results may vary due to variations among organoid populations derived from different cell types.
- Specific cell and tissue types or 3D applications may require optimization and/or process development.
- For optimal product performance and product support, product recommendations should be followed.
- Pipette tips do not need to be chilled and culture plates do not need to be pre-warmed.
- For further technical questions about the Cultrex Synthetic Hydrogel Kit, contact Bio-Techne® technical support team at techsupport@bio-techne.com for assistance.

Other Supplies Required

- Cell dissociation reagents
- Cell counting equipment
- Inverted microscope
- Vortexer
- Wide-bore pipette tips (recommended for whole organoids or non-singularized cells)
- Mini-Centrifuge
- Flat-bottom culture plate or dishes (non-tissue culture (non-TC) treated preferred)
- 37 °C, 5% CO_2 incubator
- Sterile 1X PBS
- **Optional:** Compounds for Stem Cell Proliferation & Renewal

Simplified Workflow Schematic



Baseline Procedure for Ex Vivo Organoid Culture in Cultrex™ Synthetic Hydrogel

The protocol describes embedding singularized cells in hydrogel domes using the Cultrex Synthetic Hydrogel Kit. Use as a general guide; it does not provide specific instructions for all cell types. Non-singularized cells and whole organoids can also be seeded into the hydrogel. Media formulation, optimal seeding density, non-dome cultures, and culturing timeline will vary depending on the cell type used.

Reagent Preparation

The Cultrex Synthetic Hydrogel Kit comes ready to use and should be stored in a manual defrost freezer at ≤ -70 °C. When ready to use, kit components should be gradually thawed either on ice or briefly with fingertips and immediately placed on ice once the components are completely liquid. **Do not thaw component vials in a water bath or at 37 °C.** Place components back into freezer immediately after use.

Recommended Protocol (24 well plate)

Cells designated to be cultured in the hydrogel will need to be prepared and singularized either before or during the incubation period described in Step 14. Plan accordingly to prevent cells or pre-gel mixture from sitting on ice for an extended period (>30-40 minutes for cells, >60 minutes for pre-gel mixture).

Calculating Hydrogel Volume

1. To calculate the total volume of hydrogel to prepare, determine (1) the target seeding density of cells within the gel, (2) the target volume of each individual dome, (3) the total number of domes to plate.

   Note: If plating more than 12 domes manually, it is recommended to prepare separate aliquots of hydrogel since the gel mixture will become too viscous to pipette approximately 5 minutes after adding the final component.

   Seeding Density: Optimal seeding density will depend on cell type. For intestinal organoids, it is recommended to use 15,000 cells per 10 µL dome, with 1 dome plated per well of a 24 well plate. If the optimal seeding density is unknown, start with 10,000 cells per 10 µL of hydrogel.

   Individual Dome Volume: Optimal dome size will depend on cell type and application. It is recommended to start with 10 µL domes and vary the dome size between 5 and 25 µL depending on application.

   Total Number of Domes: This will vary depending on the application or experiment.

2. Once the total volume of hydrogel has been determined, **calculate for 10-20% excess to compensate for pipette error.**

   Important: If preparing less than 50 µL of hydrogel, prepare 20% excess. If preparing 50 µL or more prepare 10% excess.

3. After calculating the total volume of hydrogel + 10-20% excess, **round up to the nearest multiple of 10 to get the final volume of gel to prepare.** This will simplify the component calculations in the next step.

4. Refer to lot-specific Certificate of Analysis to calculate the volume of each component needed: https://www.bio-techne.com/resources/cofa-finder-tool

   A. Initially, only **Components A-G** will be added to the mixture. Be sure to record the volume of **Component H** for use later in the protocol.

   Example: If preparing enough gel for six 10 µL domes (60 µL total hydrogel).

   i. **Calculate for 10% excess:** 60 µL x 1.10 = 66 µL total hydrogel

   ii. **Round up to nearest multiple of 10:** 70 µL total hydrogel

   iii. **Use CoA table to calculate necessary volumes for each component**

   Example: If Component B requires 1 µL per 10 µL of hydrogel, then 7 µL of **Component B** is required to make 70 µL total hydrogel.

Material Preparation

5. Place ice in an insulated container, and spray the container with 70% ethanol before transferring into a sterile biosafety cabinet (BSC).

6. Transfer all kit components **except Component H** from the freezer to the BSC.

7. Thaw all components briefly with fingertips, then place **Component B** at room temperature and all remaining components on ice.

   A. **Component B** may form crystalline precipitate immediately after thawing or if placed on ice. The precipitate will dissolve once warmed and vortexed.

   B. **Make sure the precipitates are dissolved** before combining with other components.

8. Once thawed, briefly vortex all components.

9. Spin down the vortexed components for 10-15 seconds in mini centrifuge to collect material at the bottom of the tubes.

Preparation of Pre-Gel Mixture

10. Add calculated volumes (from Step 4) of **Components A, B, and C** to a sterile 1.5 mL microcentrifuge tube. Mix by vortexing and briefly spin tube for 10-15 seconds in a mini centrifuge.

11. Add calculated volumes of **Components D, E, F, and G** to the same 1.5 mL tube. Mix by vortexing and briefly spin tube for 10-15 seconds in a mini centrifuge, ensuring total volume is collected at the bottom of the tube.

12. Incubate at **room temperature** in the BSC for **30 minutes.**

    A. **Immediately** after beginning incubation, return remaining kit components to the freezer and begin preparing cells.

    Note: If cells are not immediately ready, it is recommended to place the tube containing the pre-gel mixture on ice after the 30 minute incubation.

Preparation of Cells

13. Singularize cells using method of choice.

    A. When harvesting from Cultrex™ Synthetic Hydrogel, it is typically recommended using a mix of trypsin and mechanical shearing.

    Tip: After harvesting using trypsin, the cells should be washed at least once in either 1X PBS (preferred) or neutralization medium such as Advanced DMEM/F12 + 10% FBS before encapsulating in the gel. Residual trypsin on cells can interfere with gelation process.

14. Count the cells. Using the seeding density and the final volume of hydrogel calculated in Step 3 determine the number of cells needed for plating.

15. Transfer the appropriate volume of cell suspension to a 1.5 mL microcentrifuge tube or 15 mL conical tube (recommended). Place tube(s) containing cells on ice until ready to proceed to plating.

Plating Hydrogel Domes

16. Spin down cells (300 x g for 5 minutes).

17. Wash cells with 1 mL of sterile 1X PBS or neutralization media such as media (e.g. DMEM) + 10% FBS and spin down again.

    Note: Wash step removes residual trypsin and/or media from the cell pellet.

    Depending on the cell type, some media contains cysteine that can significantly interfere with formation of the hydrogel if any is left behind on the cell pellet.

18. After pelleting cells, carefully remove **all supernatant** from the pellet. Leftover supernatant can dilute and compromise hydrogel integrity.

    Tip: After removing some of the supernatant, place the tube of pelleted cells on ice for 5 minutes. This will allow residual supernatant to collect at the bottom of the tube, and it can then be pipetted off carefully.

19. Transfer non-TC treated 24 well plate to the BSC to prepare for plating.

20. Retrieve **Component H** from the freezer and transfer to the BSC. Thaw on ice or briefly with fingertips before placing tube on ice.

BEFORE PROCEEDING: Read through steps 21-26 and ensure all necessary materials are prepared. It is important to work quickly; after adding Component H, the hydrogel may become too viscous to pipette within 5 minutes.

21. **Working quickly**, re-suspend the cell pellet in the full volume of hydrogel prepared previously.

22. Mix briefly by triturating the cell/hydrogel mixture with a pipette, then vortex gently to fully re-suspend the cell pellet. **Avoid creating bubbles.**

    A. Briefly pulse the tube in a mini centrifuge to remove bubbles.

23. Add calculated volume (from Step 4) of **Component H** to the tube containing the cell/hydrogel mixture. Triturate gently and pulse vortex 3-5 times gently.

24. Carefully pipette one dome into each well.

    Tip: If plating whole organoids or non-singularized cells, use wide-bore tips for plating. Regular tips may be used when plating singularized cells.

    For best results, do not touch the bottom of the well with the pipette tip. Instead, carefully hover over the bottom of the well and gently lower the tip until the hydrogel droplet contacts the plastic.

25. Once all domes have been created, place the lid on the plate and **carefully** invert it. Flipping the plate too quickly can cause the domes to spread out or become oblong.

26. Place the plate (upside-down) in a humidified cell culture incubator (37 °C, 5% CO_2) for 15 minutes.

27. During incubation, prepare an aliquot of cell culture media in a conical tube. For a 24-well plate, it is recommended to use 600-800 µL of media per well, depending on dome size.

    A. Depending on the cell type, it is recommended to add Rock inhibitor for the first 2-3 days.

28. After the incubation, carefully flip the plate right-side-up and place it back in the incubator for another 15 minutes.

29. After the second incubation, carefully add media to each well without disturbing the domes.

    Tip: Avoid depositing media directly on top of the domes as this can damage them. Instead, gently add media along the side of each well.

30. Incubate plate in a humidified cell culture incubator (37°C, 5% CO_2). Replace media as needed depending on cell type.

    Note: When removing old media during the media exchange, use a pipette instead of an aspirator. The suction produced by the aspirator and rapid movement of the media can lead to bubbles becoming trapped underneath the hydrogel domes.

    As general guidance, the cells/organoids can be cultured in the gel as long as their size and density properties do not negatively affect the stability of the hydrogel. Monitoring these variables within the specific application of interest will help to indicate when a new gel preparation should be considered.