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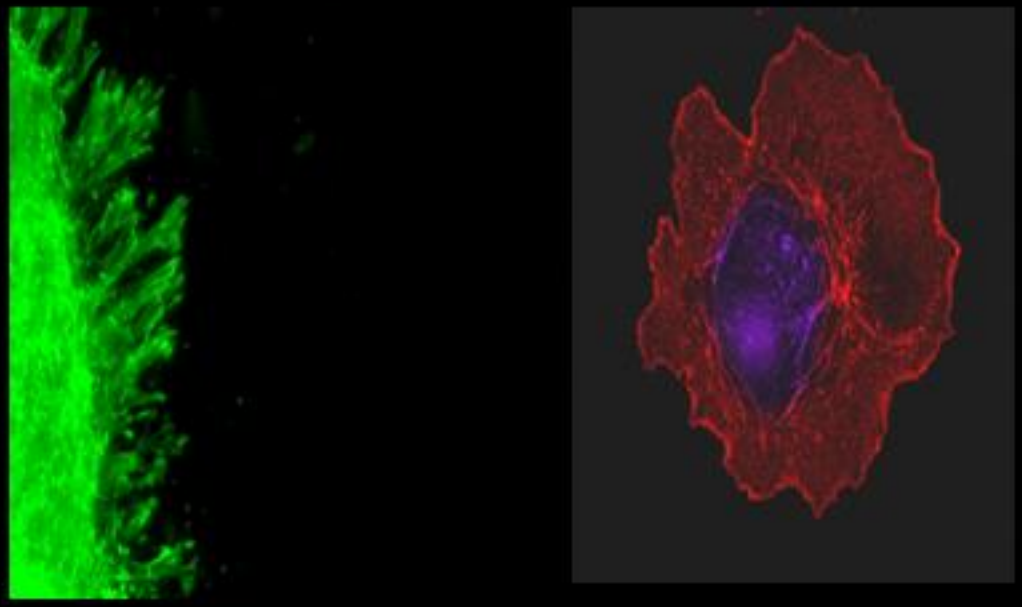
CHEMICON'S
3D Collagen Cell Culture System (ECM675)



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A simple method to analyze cell growth in a natural 3D collagen matrix

Kit Components:

- Collagen Solution
- 5X RPMI Medium
- 5X M199 Medium
- 5X DMEM Medium
- 5X PBS with Phenol Red
- Neutralization Buffer

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Materials required but not provided:

- Tissue Culture Dishes
- Pipettes and sterile tips
- Microcentrifuge tubes, sterile
- 37° CO_2 Incubator
- Inverted Microscope
- Bacterial Collagenase from *Clostridium histolyticum*
- Researchers experimental cell lines e.g. Cos-7, Hela, NIH 3T3, etc.

Advantages:

- 3D SYSTEM - Simulates collagen rich ECM of normal tissues
- TRANSLUCENT - Cell can easily be fixed and stained
- READY TO USE - All reagents are included even media, simple assay

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Preparation of Collagen Solution

- **Keep all solutions on ice or in an ice bath during the solution preparation**

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Collagen Solution Preparation

Step 1. According to Table 1, prepare the desired volume of collagen gel solution in the following sequence.

Note: 5X medium should be compatible with the desired cell type.

i. In a sterile tube, add the appropriate volume of Collagen Solution.

ii. Next, add the corresponding volume of 5X PBS or medium. Mix well. (Note: Solution should be yellow in color)

iii. Finally, add the Neutralization Solution and **immediately** mix well. (Note: Solution should change to pink / red color)

Step 2. After mixing, keep the solution on ice. The pH of the collagen gel solution should be neutral, which is indicated by the pink / red color of phenol red in the 5X medium.

Total Volume (mL)	Collagen Solution (mL)	5X PBS or Medium (mL)	Neutralization Solution (µL)
10	8	2	250
5	4	1	125
2.5	2	0.5	62.5
1	0.8	0.2	25
0.5	0.4	0.1	12.5

Table 1: Preparation of collagen gel solution

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Step 1. Harvest and resuspend desired cell line at 0.1 to 0.2 x 10^6 cells/mL.

Culturing cells in the Collagen Gel:

Step 2. Mix a small volume of desired cell suspension with the chilled collagen solution under sterile conditions.
<u>Note:</u> Cell Suspension should not be greater than 10% of the final volume of collagen solution.

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Step 3. Add the proper volume of the chilled collagen solution (containing cells) onto a tissue culture plate or dish. See Table below for recommended volumes.

Culture Dish	96-well	48-well	24-well	6-well	35mm	60mm	100mm
Volume (mL)	0.1	.02	0.5	1.0	2.0	3.0	5.0

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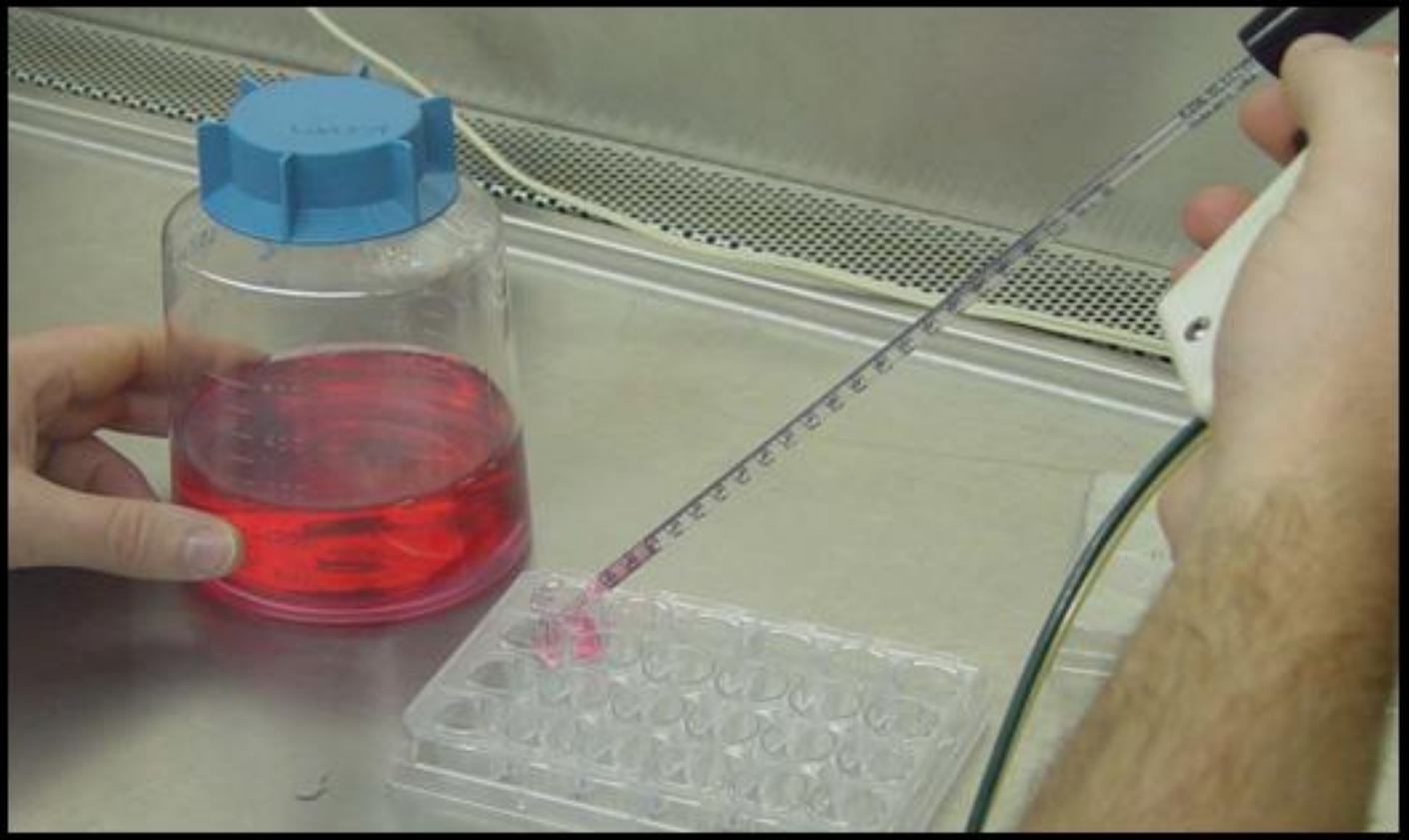
Step 4. Immediately transfer to 37°C for 60 min to initiate polymerization of the collagen. The polymerized gel will look cloudy.

Step 5. After formation of the collagen gel, add culture medium to cover the collagen gel.



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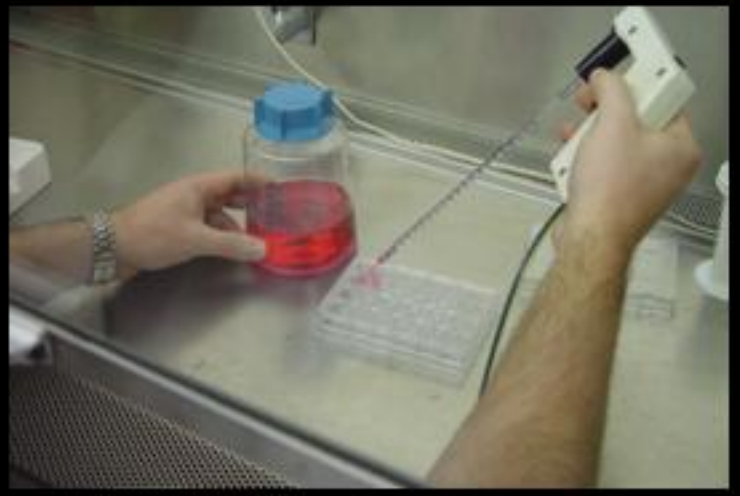
Step 6. Incubate cells overnight or several days at 37°C with CO_2 as experiments require. Change culture medium daily.



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Step 7. Cells can be visualized using an inverted or phase contrast microscope and / or can be directly fixed and stained within the collagen.

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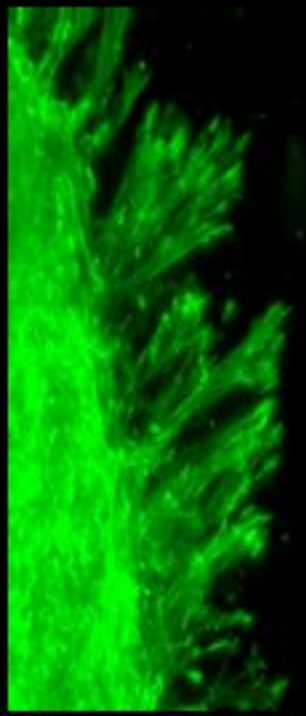
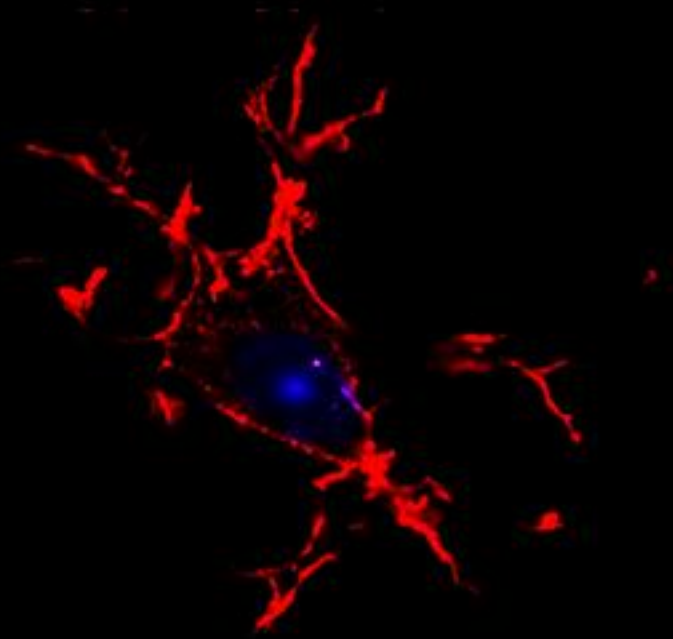
3D Collagen Cell Culture results below:



Chick embryonic fibroblast cells migrating into 3D collagen.



Confocal image of Cos-7 actin cytoskeleton in 3D collagen.