3D Cell Culture Gel for tumor cell, stem cell and primary cell

Cat. #:

Cat.#	Stiffness	Volume	Strength	3 Stiffness Application
P720S-2	Soft	2 mL	0.9 ~ 1.5 kPa	Circulating cells, Nerve cells, BT474, HL60, P39
P720S-10	Soft	10 mL		
P720M-2	Med	2 mL	14 ~ 20 kPa	Muscle cells, Adipose cells, C2C12, HepG2
P720M-10	Med	10 mL		
P720H-2	Stiff	2 mL	35 ~ 47 kPa	Bone cells
P720H-10	Stiff	10 mL		
P720SMH-6	S/M/H	2 mL x 3	Soft, Med, Stiff, 2 mL each for customer testing their cells	

Customer can mix any two gels at any ratio to make their own special stiffness gel.

Application:

3D gel is a tailorable 3D cell culture matrix for **tumor cell, stem cell** and **primary cell**.

It provides collagen backbone for cell attachment and allows customizing matrix components by additional extracellular matrix, growth factors, cytokines, or hormones for optimizing three dimensional cell culture.

This product is for research use only.

Shipping / Storage:

Ship at 4°C. Store Component A at 4˚C, and Component B at -80˚C immediately upon receiving. Avoid freeze-thaw cycle.

Shelf Life:

Component A can be preserved at 4˚C for up to one year. Do not freeze.
Component B can be preserved at -80˚C for up to 9 months, at -20˚C for up to 3 months.

The reconstituted Component B (dissolved into ddH_2O) can be preserved at -20˚C for up to 3 months, and at 4˚C for up to 1 month.

Components:

Component	P720S-2 P720M-2 P720H-2	P720S-10 P720M-10 P720H-10	P720SMH-6	Storage
Component A	2 mL	2 mL x 5 vials	2 ml x 3 vials (Soft, Med, Stiff, 2 mL each)	4°C Do not freeze.
Component B	1 vial	5 vials	3 vials	–20°C for 3 months –80°C for 9 months

Product description

3D cell culture gel is a tailorable collagen-based hydrogel that formed solid substrate after cross-linking. It is a promising biomaterial overcomes many limitations imposed by other substrate systems. Unlike other substrate systems, 3D cell culture gel is operated at room temperature. It not only can be used a carrier for cell or drug delivery, but also *in vitro* cell culture platforms. Besides, it contains cell essential nutrients for cell survival, and provides a stable and durable platform for long-term studies such as cell-cell, cell-matrix interactions and etc. Moreover, cells can either be seeded on top of gel (2D) or embedded inside the gel (3D). The size of 3D cell culture gel can be modified for different assays. Other matrix proteins, growth factors, hormones or cytokines can be included depending on the experimental design. The biocompatibility and excellent transparency of the 3D cell culture gel allow direct observation under a light microscope, and direct staining to avoid tedious steps such as sectioning and antigen retrieval. Other protein, RNA and DNA assays can also be performed to characterize the encapsulated cells in 3D cell culture gel. For different cell types, 3D cell culture gel is available in three different stiffness (i.e., Soft, Med and Stiff) with varies matrix

yield strength ranging from 1kPa to 40kPa. Figure 1 demonstrates the recommended 3D cell culture gel matrix for different cell types.

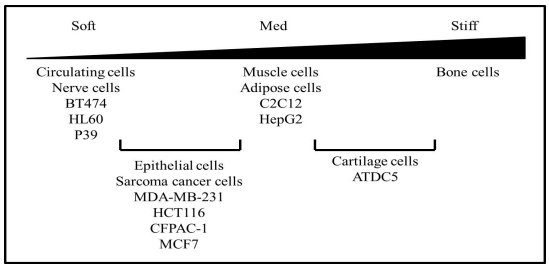

Figure 1: Recommended 3D cell culture gel matrix for different cell types.

Additional Required Instruments or Material

- 0.25% trypsin
- Phosphate buffer saline (PBS)
- Benchtop microcentrifuge
- 37˚C water bath tank
- Sterilized 2mL centrifuge tubes
- 48- well- suspension plate (without tissue culture treated) or other none tissue culture treated dishes. (Purchased from Greiner Bio-One, Cat.677102, VWR Cat.25384-088, or VWR Cat.25384-090)
- Medium: Cell culture medium supplemented with 10% serum
- Autoclaved, distilled water

Protocol

Sample preparation

1. Prepare the required materials (see above).
2. Thaw Component A at 55˚C and Component B at room temperature. ***Note:*** *Do not open Component A and Component B outside sterilized cell culture hood.*
3. Briefly spin Component vial before open the cap. Component B is lyophilized white powder. Add **100 µL** autoclaved, distilled H_2O to **one Component B vial** to reconstitute the Component B. Mix well by vortex and inverting the vial, till all white powder is dissolved. Quick spin to collect the solution at the bottom of the vial.

Three Models (2D culture, 3D culture or *in vivo* delivery)

A. *In vitro* 2D cell culture (use as culture surface coating)

1. Mix 100 µL Component B with 2mL Component A.
2. Pour evenly on 48-well tissue culture plate or tissue culture treated dish.
3. Incubate plate/dish at 37˚C for 45 minutes.
4. Upon gel solidified, detach cells with 0.25% trypsin from 80% confluent cell culture dishes or flasks.
5. Neutralize trypsin with Medium.
6. Count cells and determine the required experimental cell number. Evenly distribute the desired amount of cells on the gel coated area evenly.

B. *In vitro* 3D cell culture

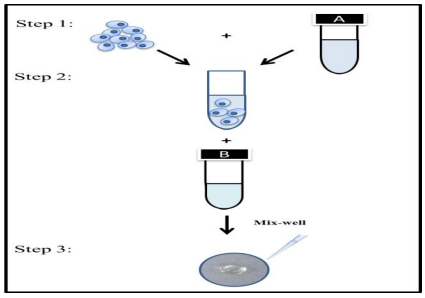

Figure 2: Scheme of 3D cell culture gel 3D culture protocol.

1. Detach cells with 0.25% trypsin from 80% confluent cell culture dishes or flasks.
2. Neutralize trypsin with Medium.
3. Count cells and determine the required experimental cell number. The suggested cell density is $2x10^6$ cells/mL of gel. Transfer the desired amount of cells into 2mL centrifuge tube and centrifuge to precipitate the cells.
4. Wash cells with Medium twice. Discard medium and mix the cells with Component A through gentle pipetting (avoid bubbles). Add other experimental factors such as matrix proteins or cytokines at this step if applicable.
5. Add reconstituted Component B into the mixture and mix well through gentle pipetting (Component B : A = 1 :20). *Note: Component B should be added at the last step prior seeding as the crosslinking reaction is irreversible.*
6. Aliquot 1-100 µL (recommended to start with 20 µL) 3D cell culture gel/cells mixture into each well of the 48- well suspension plate or other non-tissue culture treated dish.
7. Incubate plate/dish at 37˚C for 45 minutes. Note that 3D cell culture gel will provide sufficient hydration for sustaining the encapsulated cells up to 8 hours.
8. Upon gel solidification, add cell specific culture medium to cover the gel, and keep culturing.
9. For cell analysis assays
    a. Characterization
        i. Fix samples with 10% neutral buffered formalin or other fixation solutions for 15 minutes.
        ii. Wash twice with PBS.
        iii. Follow the standard staining protocol for the remaining step(s).
    b. DNA, RNA, and protein extraction
        i. Wash with PBS twice.
        ii. Release cells from 3D cell culture gel matrix with 0.25% trypsin at 37˚C (~ 4 hours).
        iii. Centrifuge to precipitate the cells.
        iv. Wash cells twice with Medium.
        v. Discard medium and add cell lysis buffer or RNA extraction buffer for DNA, RNA or protein extraction.
        vi. Follow the standard extraction protocols for the remaining step(s).

C. *In vivo* cell delivery

D.

1. Detach cells with 0.25% trypsin from 80% confluent cell culture dishes or flasks.
2. Neutralize trypsin with Medium.
3. Count cells and determine the desired cell number. The suggested cell density for tumor induction is $2x10^6$ cells /100µL of gel.
4. Transfer the desired amount of cells into 2mL centrifuge tube and separate cells from solution through centrifugation.
5. Wash twice with Medium.
6. Discard medium and mix the cells well with Component A through gentle pipetting (avoid bubbles). Add other experimental factors such as matrix protein or cytokines at this step if applicable.

7. Add proper volume of reconstituted Component B into the mixture and mix well through gentle pipetting (Component B : A = 1 :20). Transfer samples into 1mL syringes immediately after mixing. *Note: Component B should be added at the last step prior delivery as the crosslinking reaction is irreversible. The crosslinking reaction takes about 15-45minutes.*
8. Apply 27G or smaller needle for delivering the prepared sample to the treatment site.

Troubleshooting

Problem	Solution
Unable to solidify	Increase dose of Component B Wash the cells one more time Use fresh Component A and Component B Reduce mixing materials
Solidify too fast	Reduce dose of Component B
Cell unable to proliferate in the matrix	Use other category of Component A Add other matrix proteins into Component A
Hard to observe under microscope	Reduce cell density Use phenol red-free medium

References

1. Kuwahara, Kenrick, Zhi Yang, Ginger C. Slack, Marcel E. Nimni, and Bo Han. "Cell delivery using an injectable and adhesive transglutaminase–gelatin gel."*Tissue Engineering Part C: Methods* 16, no. 4 (2009): 609-618.
2. Kuwahara, Kenrick, Josephine Y. Fang, Zhi Yang, and Bo Han. "Enzymatic crosslinking and degradation of gelatin as a switch for bone morphogenetic protein-2 activity." *Tissue Engineering Part A* 17, no. 23-24 (2011): 2955-2964.
3. Fang, Josephine, Zhi Yang, ShihJye Tan, Charisse Tayag, Marcel E. Nimni, Mark Urata, and Bo Han. "Injectable gel graft for bone defect repair."*Regenerative medicine* 9, no. 1 (2014): 41-51.
4. Tan, ShihJye, Josephine Y. Fang, Zhi Yang, Marcel E. Nimni, and Bo Han. "The synergetic effect of hydrogel stiffness and growth factor on osteogenic differentiation." *Biomaterials* 35, no. 20 (2014): 5294-5306.
5. Fang, Josephine Y., Shih-Jye Tan, Zhi Yang, Charisse Tayag, and Bo Han. "Tumor Bioengineering Using a Transglutaminase Crosslinked Hydrogel." *PloS one* 9, no. 8 (2014): e105616. – END -
6.

Our customers also buy:

Water Bath Treatment	1 tablet / treatment / week Cat.# T20
Simple, Reliable	SV40T / hTERT *Cell Immortalization Kits*