3D Cell Culture Gel (Col-Tgel) for tumor cell, stem cell and primary cell

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

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

**Application:**
Col-Tgel 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 ddH₂O) can be preserved at -20°C for up to 3 months, and at 4°C for up to 1 month.

**Components:**

<table>
<thead>
<tr>
<th>Component</th>
<th>P720S-2</th>
<th>P720M-2</th>
<th>P720S-10</th>
<th>P720M-10</th>
<th>P720H-10</th>
<th>P720SMH-6</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component A</td>
<td>2 mL</td>
<td>2 mL x 5 vials</td>
<td>2 mL x 3 vials</td>
<td>(Soft, Med, Stiff, 2 mL each)</td>
<td>4°C</td>
<td>Do not freeze.</td>
<td></td>
</tr>
<tr>
<td>Component B</td>
<td>1 vial</td>
<td>5 vials</td>
<td></td>
<td></td>
<td></td>
<td>3 vials</td>
<td>-20°C for 3 months -80°C for 9 months</td>
</tr>
</tbody>
</table>

**Product description**
Col-Tgel 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 system, Col-Tgel 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 Col-Tgel 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 Col-Tgel allow a 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 Col-Tgel. For different cell types, Col-Tgel is available in three different stiffness (i.e., Soft, Med and Stiff) with varies matrix yield strength ranged from 1kPa to 40kPa. Figure 1 demonstrates the recommended Col-Tgel 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 H₂O 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 Col-Tgel 3D culture protocol.](image)

**Figure 1:** Recommended Col-Tgel matrix for different cell types.
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 \(2 \times 10^6\) 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). \textit{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) Col-Tgel/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 Col-Tgel 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 Col-Tgel 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. \textit{In vivo} cell delivery

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 \(2 \times 10^6\) 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. \textit{Note: Component B should be added at the last step prior delivery as the crosslinking reaction is irreversible. The crosslinking reaction takes about 15-45 minutes.}
8. Apply 27G or smaller needle for delivering the prepared sample to the treatment site.
### Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Solution</th>
</tr>
</thead>
</table>
| 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


### Our customers also buy:

<table>
<thead>
<tr>
<th><strong>Better than Matrigel</strong></th>
<th><strong>Real 3D</strong> cell culture gel, 3-stiffness &amp; save  Cat.# P720</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse Tail DNA Extract</strong></td>
<td>20 min. done. Accurate &amp; consistent. No use of organic solvents. Ready for PCR  Cat.# T605</td>
</tr>
<tr>
<td><strong>1 drop, do PCR Mix</strong></td>
<td>PCR Master Mix - easy dripping  Cat.# W2599-5</td>
</tr>
<tr>
<td><strong>10X Virus Titer-up Boost</strong></td>
<td>Regulates packaging in transcriptional level Increases Lenti/Retro viral particles up to 10-fold  Cat.# P906 / P909</td>
</tr>
<tr>
<td><strong>Water Bath Treatment</strong></td>
<td>1 tablet / treatment / week  Cat.# T20</td>
</tr>
<tr>
<td><strong>Simple, Reliable</strong></td>
<td>SV40T / hTERT Cell Immortalization Kits</td>
</tr>
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