**VitroGel™ 3D is an animal origin-free polysaccharide hydrogel system**

Closely mimicking the natural extracellular matrix (ECM) environment, this distinct system brings many advantages to bridge *in vitro* and *in vivo* studies through 3D cell culture and beyond:

- Perform procedure at room temperature with a simple mixing step.
- Compatible with imaging and downstream analysis.
- Injectable for *in vivo* studies.

**Ready-to-use**
The hydrogel system is room temperature stable with neutral pH. Just mix with your cells and you are DONE!

**Fast gelation**
Gelation starts immediately right after mixing and becomes stable in 15 minutes. Cells distribute homogeneously in the hydrogel.

**Transparent**
The hydrogel is transparent and compatible to different imaging systems for cell observation.

**Permeable**
Oxygen, nutrition and other molecules can easily move in/out the hydrogel system. Great for drug discovery studies!

**Cell harvesting**
After 3D cell culture, cells can be easily harvested from the hydrogel by using standard centrifuging methods.

**Injectable**
Using the right mixing ratio, the hydrogel becomes injectable. Great for *in vivo* studies!

**Enjoy VitroGel 3D with great benefits**

- **Save time:** Simply mixing the hydrogel solution with cell culture media for an ECM mimicking environment. No additional reagents or preparation steps are needed.

- **Easy operation:** The VitroGel 3D hydrogel system is ready-to-use at room temperature. After mixing for hydrogel formation, the system can be simply transferred by pipetting.

- **Cost saving:** Each VitroGel 3D (10 mL/vial) is good for 2.5 96-well plates at standard mixing ratio. Downstream analysis can be done with standard protocols and reagents.

- **More accurate:** The morphologies and behaviors of cells grown in VitroGel 3D hydrogel system are similar to their natural state, providing data to bridge *in vitro* and *in vivo* studies.

- **Multiple applications:** The VitroGel 3D hydrogel system has great flexibilities of physical/chemical properties and the hydrogel preparation can also be manipulated to meet different applications.
How does it work?

Stage One
A free flowing hydrogel solution
The polyanhydride molecules are stable as short nanofiber structures at room temperature and ready-to-use for hydrogel formation by mixing with an ionic solution.

Stage Two
An injectable hydrogel
To make an injectable hydrogel, we recommend mixing hydrogel solution 1 with cell medium 2 at a 4:1 ratio (1:2; 1:2 = 1:1 v/v, for best cell culture media and PBS).

Stage Three
A firm hydrogel
After transferring the hydrogel to the desired container/location, adding additional ionic solution 3, such as cover the hydrogel with extra cell culture media, would bundle nanofiber parallel 4 for a firm shaped hydrogel.

VitroGel 3D vs other 3D cell culture methods

<table>
<thead>
<tr>
<th></th>
<th>VitroGel 3D</th>
<th>Basement membrane matrix</th>
<th>Polymer matrix</th>
<th>Hanging drop plate</th>
<th>Low adhesion plate</th>
<th>Micro-patterned plate</th>
<th>Magnetic Levitation</th>
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<tbody>
<tr>
<td>Ready-to-use</td>
<td>✔</td>
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<td>Mimic natural ECM</td>
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<td>No undesired growth factors</td>
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<tr>
<td>Room temperature operation</td>
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<tr>
<td>Neutral pH</td>
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<td>Cell harvesting</td>
<td>✔</td>
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<tr>
<td>Transparent</td>
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<td>Modifiable for cell adhesion</td>
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<td>Control hydrogel stiffness</td>
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<td>Injectable</td>
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Multiple applications of VitroGel™ 3D

Bridge the in vitro and in vivo studies by creating natural cellular environment

Control the stiffness of substance cell attached, study cell invasion, migration and more

Injectable hydrogel property for in vivo studies, cell harvesting after 3D cell culture and other applications

Case Study

VitroGel 3D has been successfully used on different projects with many cell lines such as human and mouse pancreatic beta cells, insulin secreting beta cell derived lines, lymphocytes, Hela cells, human embryonic kidney 293 cells, human colon carcinoma cell lines (HCT–8), breast cancer cells and much more. The results show that cells can suspend homogeneously in the hydrogel and successfully grow in the 3D structure. Cell culture media can easily penetrate the hydrogel matrix and provide nutrition for long-term cell culture. The growth of cells can be observed easily under microscopy.

Case 1: Beta TC3 cells
- Application: 3D cell culture
- Product: VitroGel 3D-RGD
- Seeding number: 5×10^5 cells/mL
- Time: 14 days

Case 2: Ins-1 cells
- Application: 3D cell culture
- Product: VitroGel 3D-RGD
- Seeding number: 5×10^5 cells/mL
- Time: 14 days
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<th>Description</th>
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<td>BCTWC001</td>
<td>Vitroge 3D Ready-To-Use Hydrogel</td>
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<tr>
<td>BCTWC002</td>
<td>Vitroge 3D-RGD Ready-To-Use Hydrogel</td>
<td>10 ml</td>
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<td>Vitroge 3D Ready-To-Use Hydrogel - sample</td>
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<tr>
<td>BCTWC002s</td>
<td>Vitroge 3D-RGD Ready-To-Use Hydrogel - sample</td>
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