



VitroGel®

High Concentration Hydrogels

tunable hydrogels

Cat No: TWG001 VitroGel® 3D High Concentration
Cat No: TWG003 VitroGel® RGD High Concentration
Cat No: TWG007 VitroGel® IKVAV High Concentration
Cat No: TWG008 VitroGel® YIGSR High Concentration
Cat No: TWG009 VitroGel® COL High Concentration
Cat No: TWG010 VitroGel® MMP High Concentration

Handbook

Rev. 4.0 July 2021

Check our website for latest protocol guideline revision

www.thewellbio.com/protocols

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TheWell
BIOSCIENCE

Growing Cells in New Dimensions

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PRODUCT DESCRIPTION AND SPECIFICATIONS

VitroGel® High Concentration hydrogel kits are our xeno-free, tunable hydrogels for researchers wanting full control to manipulate the biophysical and biological properties of cell culture environment. The tunability of the hydrogel gives the ability to create an optimized environment for cell growth. The solution transforms into a hydrogel matrix by simply mixing with the cell culture medium. No cross-linking agent is required. Cells cultured in this system can be easily harvested. The hydrogel is also injectable for *in vivo* studies. From 2D coating, 3D culture to animal injection, VitroGel makes it possible to bridge the *in vitro* and *in vivo* studies with the same platform system.

All VitroGel® High Concentration hydrogel kits come with a high concentration VitroGel solution and a VitroGel® Dilution Solution. The VitroGel Dilution Solution is a ready-to-use solution for mixing with the VitroGel system to adjust the hydrogel concentration for different hydrogel strengths. There are two types of VitroGel Dilution Solution to select from:

- TYPE 1: Contains sucrose to maintain the best osmolarity.
- TYPE 2: Sucrose free formulation for cells sensitive to the sugar level in the medium.

Catalog No.	Name	Description
TWG001	VitroGel® 3D High Concentration	Unmodified synthetic hydrogel
TWG003	VitroGel® RGD High Concentration	RGD peptide high concentration modified
TWG009	VitroGel® COL High Concentration	Collagen-mimetic functional hydrogel
TWG010	VitroGel® MMP High Concentration	Matrix metalloproteinases (MMP) biodegradable
TWG007	VitroGel® IKVAV High Concentration	IKVAV modified
TWG008	VitroGel® YIGSR High Concentration	YIGSR modified

Sizes	3 mL hydrogel with 50 mL Dilution Solution
Expiration	24 months from manufacture
Storage conditions	Store 2°C to 8°C. DO NOT FREEZE.
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MULTIPLE CELL CULTURE METHODS WITH VITROGEL HYDROGELS

The XENO-FREE bio-functional VitroGel hydrogel system is versatile for many cell culture methods and applications. Choose either the “ready-to-use” hydrogel system for an optimized formulation and a simple operating process or the high-concentration hydrogel system to create a customized microenvironment by “Mix & Match” and tuning of the hydrogels. There are multiple ways to use our hydrogel system to fulfill your cell culture research needs.

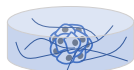
To show the flexibility of our hydrogels, we list five of the most popular cell culture methods that can be performed with our hydrogel:

- 3D Cell Culture
- 2D Hydrogel Coating
- Static Suspension Culture
- Hydrogel-Cell Bead
- Injectable Carrier

These five culture methods apply to all our ready-to-use VitroGel and high concentration VitroGel systems. Cells cultured in these methods can be easily harvested with the VitroGel® Cell Recovery Solution for downstream analysis or subculture.

3D CULTURE

Encapsulate cells in the hydrogel matrix to promote cell-matrix and cell-cell interactions.



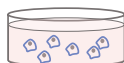
2D HYDROGEL COATING

Control the substance properties for culturing cells on top of the hydrogel. Great for cell submergence or invasion studies.



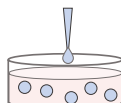
STATIC SUSPENSION CULTURE

Change the viscosity of the cell medium to create a cell suspension environment for large-scale cell expansion.



HYDROGEL-CELL BEAD

Encapsulate cells in the hydrogel matrix by creating hydrogel-cell droplets.



ANIMAL INJECTION

Mix compounds or cells with the injectable hydrogel to increase cell retention and viability for *in vivo* studies.



Learn more about 3D Cell Culture Methods with VitroGel:

www.thewellbio.com/3d-cell-culture-hydrogel/3d-cell-culture-methods-with-vitrogel/

3D CELL CULTURE

Video Protocol Online

thewellbio.com/video-protocols

Materials:

- VitroGel High Concentration
- Cells
- Cell culture medium
- VitroGel Dilution Solution (CAT# MS01-50 or MS02-50)
- Micropipette; Low retention pipette tips
- Tissue culture treated plate

 Note: Non-tissue culture treated well plate may cause hydrogel detachment. If using a glass bottom plate, please use tissue culture treated glass bottom plate or Poly-D-Lysine coated glass bottom plate for a better hydrogel attachment.

PROTOCOL

Read **3D CELL CULTURE AND 2D COATING HELP GUIDE** on page 16 for protocol tips carefully.

1. Bring VitroGel to room temperature and warm cell culture medium to 37° C if needed.
2. Adjust the concentration of VitroGel for different cell types by diluting the VitroGel with VitroGel Dilution Solution. After dilution, gently mix the diluted VitroGel with a cell suspension (in the desired media) without introducing bubbles. Recommend cell concentration of $0.5\text{-}2 \times 10^6$ cells/mL. See Table 1.1 below for solution/medium volume of different dilutions

Table 1.1: Volumes of solution/medium for different hydrogel dilutions for 3D cell culture (each well of a 24-well plate)

Dilution Ratio	VitroGel	VitroGel Dilution Solution	Cell Medium w / Cells
1:0	240 μ L	0 μ L	60 μ L
1:1	120 μ L	120 μ L	60 μ L
1:2	80 μ L	160 μ L	60 μ L
1:3	60 μ L	180 μ L	60 μ L
1:5	40 μ L	200 μ L	60 μ L

If cells are to be cultured in complete cell culture medium with 10% FBS or other critical growth factors/supplement, prepare the cell suspension by following the step below:

- a. Prepare 100% FBS with 10X of critical growth factors.
- b. Prepare cells in regular 1X cell culture medium. (Do not make the medium at a high concentration as the ionic molecules would affect the hydrogel formation.)

- c. Mix the solution from step a) and b) to get cell suspension in 50% FBS with 5X critical growth factors.
- d. Mix the diluted VitroGel with cell suspension at 4:1 v/v ratio (eg.400 μ L diluted VitroGel with 100 μ L cell suspension).

Note: If the cells need to culture at a higher FBS concentration (eg. 20%), prepare cells suspension directly in 100% FBS. Prepare the diluted VitroGel by mixing VitroGel with VitroGel Dilution Solution and wait 30-60 min before mixing it with cell suspension. Wait 20-30 min at room temperature (or 37°C) before adding the cover medium on top.

3. Transfer the hydrogel mixture to a well plate. Gently tilt/swirl the well plate to ensure there is an even coating on the bottom of each well.

⚠ Note: Adding the hydrogel as a dome instead of covering the whole bottom of the well plate might cause hydrogel detachment.

The recommend volume of each well/insert is listed in Table 1.2 and Table 1.3 below:

Table 1.2: Recommended hydrogel volume for WELL PLATES

WELL PLATE	Volume of hydrogel (μ L)	Volume of Cover Medium (μ L)
6 -well plate	1200	1200
12-well plate	600	600
24 well plate	300	300
48-well plate	150	150
96-well plate	75	75

Table 1.3: Recommended hydrogel volume for PLATE INSERTS

PLATE INSERTS	Volume of hydrogel (μ L)	Volume of Cover Medium (μ L)
6 -well plate	800	800
12-well plate	400	400
24 well plate	200	200
48-well plate	100	100
96-well plate	50	50

4. **Wait 10-20 min at room temperature for a soft gel formation.**

Note: During the hydrogel forming process, do not disrupt the hydrogel by tilting or shaking the well plate.

5. **After soft gel formation, GENTLY tilt the well plate to check if hydrogel has formed and attached firmly to the bottom of the well plate.**
6. **Carefully cover hydrogel with additional medium to further stabilize the hydrogel.** See Table 5 or Table 6 for recommended volume of cover medium.
7. **Place the well plate in an incubator and change the cover medium every 48 hours.**

Note: We recommend to only change 60-80% of the top medium without disturbing the hydrogel.

2D COATING

Materials:

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- VitroGel High Concentration hydrogel
- Cells
- Cell Culture medium
- VitroGel Dilution Solution (CAT# MS01-50 or MS02-50)
- Micropipette; Low retention pipette tips
- Tissue culture treated plate

⚠ Note: Non-tissue culture treated well plate may cause hydrogel detachment. If using a glass bottom plate, please use tissue culture treated glass bottom plate or Poly-D-Lysine coated glass bottom plate for a better hydrogel attachment.

PROTOCOL

Read **3D CELL CULTURE AND 2D COATING HELP GUIDE** on page 16 for protocol tips carefully.

1. Bring VitroGel to room temperature and warm cell culture medium to 37°C if needed.
2. Gently mix VitroGel with cell culture medium without introducing bubbles. If needed, adjust the concentration of VitroGel by diluting with VitroGel Dilution Solution. See Table 2.1 below for solution/medium volume of different dilutions.

Table 2.1: Volumes of solution/medium for different hydrogel dilutions for 2D coating (each well of a 24-well plate)

Dilution Ratio	VitroGel	VitroGel Dilution Solution	Cell Medium for Mixing
1:0	240 µL	0 µL	60 µL
1:1	120 µL	120 µL	60 µL
1:2	80 µL	160 µL	60 µL
1:3	60 µL	180 µL	60 µL
1:5	40 µL	200 µL	60 µL

(The diluted VitroGel coating can also be prepared by mixing VitroGel with VitroGel Dilution Solution (without cell medium) and transferring the hydrogel mixture to a well plate and leave overnight.)

The 2D coating hydrogel can be enriched by mixing with FBS and other critical growth factors/supplement: Adding FBS and growth factors to the 2D coating hydrogel can improve the cell attachment and growth. To prepare 2D coating hydrogel with FBS:

- a. Add 5X concentration of FBS and critical growth factors to 1X cell culture medium (Do not make the medium at a high concentration as the ionic molecules would affect the hydrogel formation)
- b. Mix the diluted VitroGel with medium above at 4:1 v/v ratio (eg. 400 μ L diluted VitroGel with 100 μ L medium)

Note: If 2D coating gel with a higher FBS concentration (eg. 20% final FBS) is needed, prepare the diluted VitroGel by mixing VitroGel with VitroGel Dilution Solution and wait 30-60 minutes before mixing it with 100% FBS. Wait 30 minutes (or overnight) at room temperature or at 4°C before adding the cells on top.

3. **Transfer the hydrogel mixture to a well plate. Gently tilt/swirl the well plate to ensure there is an even coating on the bottom of each well.**

⚠ Note: Adding the hydrogel as a dome instead of covering the whole bottom of the well plate might cause hydrogel detachment.

The recommend volume of each well is listed in the table 2.2 and 2.3 below:

Table 2.2: Recommend 2D coating hydrogel volume for WELL PLATE

WELL PLATE	Volume of hydrogel (μ L)	Volume of cells (μ L)
6-well plate	1200	1200
12-well plate	600	600
24-well plate	300	300
48-well plate	150	150
96-well plate	75	75

Table 2.3: Recommend 2D coating hydrogel volume for PLATE INSERT

PLATE INSERT	Volume of hydrogel (μ L)	Volume of cells (μ L)
6-well plate	800	800
12-well plate	400	400
24-well plate	200	200
48-well plate	100	100
96-well plate	50	50

4. **Wait 10-20 min at room temperature for a soft gel formation.**

Note: During the hydrogel forming process, do not disrupt the hydrogel by tilting or shaking the well plate.

5. **After soft gel formation, GENTLY tilt the well plate to ensure the hydrogel is formed and attached firmly to the bottom of the well plate. Carefully add cell culture medium with cells to cover hydrogel.**
Recommend cell concentration of $1-5 \times 10^5$ cells/mL.

6. Place the well plate in an incubator and change the cover medium every 48 hours.

Note: We recommend to only change 60-80% of the top medium without disturbing the hydrogel.

STATIC SUSPENSION CULTURE

Materials:

Video Protocol Online

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- VitroGel® High Concentration
- Cells
- Cell culture medium
- VitroGel Dilution Solution (Cat# MS01-50 or MS02-50)
- Additional supplement (optional)
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- Cell culture vessel (plate, flask, bioractor)

PROTOCOL

1. Bring VitroGel hydrogel to room temperature or warm at 37°C.
2. Prepare cell suspension in the cell culture medium.
 - Recommended cell concentration $0.5-2 \times 10^6$ cells/mL.
 - **Optional:** To control the critical growth factors/inhibitors/serum in hydrogel, add desired supplement in cell suspension at 5X concentration. The cell suspension then can mix with VitroGel hydrogel solution to get 1X final concentration in Step 3.
3. Adjust the concentration of VitroGel for different cell types by diluting the VitroGel with VitroGel Dilution Solution. After dilution, mix the diluted VitroGel solution with a cell suspension from Step 2 for hydrogel-cell mixture. Gently pipette up and down 5-10 times to mix thoroughly without introducing bubbles. See Table 3.1 below for solution/medium volume of different dilutions.

Table 3.1: Volumes of solution/medium for different hydrogel dilutions for static suspension culture

Dilution Ratio	VitroGel	VitroGel Dilution Solution	Cell medium w/ Cells
1:0	800 µL	0 µL	200 µL
1:1	400 µL	400 µL	200 µL
1:2	200 µL	400 µL	200 µL
1:3	200 µL	600 µL	250 µL
1:4	200 µL	800 µL	250 µL
1:5	100 µL	500 µL	150 µL

4. Disperse the hydrogel-cell mixture from step 3 in cell culture medium by mixing them at 1:1 to 1:10 v/v ratios for a hydrogel-cell suspension (e.g. mix 1 mL of hydrogel-cell mixture with 3 mL cell culture medium for 1:3 mixing). Carefully pipette up and down to mix homogeneously.

Note: the final viscosity of the hydrogel-cell suspension can be adjusted by changing the mixing ratio between hydrogel-cell mixture and cell culture medium to fulfill the culture conditions of various cell types, cell seeding density and culture vessels. Usually the 1:1 to 1:5 mixing ratio can main good hydrogel-cell suspension without plate shaker or agitation. For mixing ratio over 1:5, the hydrogel can help to maintain a great cell suspension under low agitation speed (10-40 rpm) to reduce shearing force and promote cell growth.

5. Transfer the hydrogel-cell suspension to a culture vessel for incubation.

HYDROGEL-CELL BEAD

Materials:

Video Protocol Online

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- VitroGel® High Concentration
- Cells
- Cell culture medium
- VitroGel Dilution Solution (Cat# MS01-50 or MS02-50)
- Additional supplement (optional)
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- Cell culture vessel (plate, flask, bioractor)

PROTOCOL

1. Bring VitroGel hydrogel to room temperature or warm at 37°C.
2. Prepare cell suspension in the cell culture medium.

Recommended cell concentration 0.5-2 x 10⁶ cells/mL.

Optional: To control the critical growth factors/inhibitors/serum in hydrogel, add desired supplement in cell suspension at 5X concentration. The cell suspension then can mix with VitroGel hydrogel solution to get 1X final concentration in step 3.

3. Adjust the concentration of VitroGel for different cell types by diluting the VitroGel with VitroGel Dilution Solution. After dilution, mix the diluted VitroGel solution with a cell suspension from step 2 for hydrogel-cell mixture. Gently pipette up and down 5-10 times to mix thoroughly without introducing bubbles. See Table 4.1 below for solution/medium volume of different dilutions.

Table 4.1 Volumes of solution/medium for different hydrogel dilutions for static suspension culture

Dilution Ratio	VitroGel	VitroGel Dilution Solution	Cell medium w/ Cells
1:0	800 µL	0 µL	200 µL
1:1	400 µL	400 µL	200 µL
1:2	200 µL	400 µL	200 µL
1:3	200 µL	600 µL	250 µL
1:4	200 µL	800 µL	250 µL
1:5	100 µL	500 µL	150 µL

4. Add cell culture medium to a well plate. The recommended volumes of cell medium for specific well plate types are listed below.

	6 well plate	12 well plate	24 well plate	48 well plate	96 well plate
Volume per well	3000 μ L	1500 μ L	750 μ L	300 μ L	10 μ L

5. Using a pipettor with a 100 μ L tip, carefully pipette the hydrogel-cell mixture from step 3 into the well plate as droplets. (roughly 5-10 droplets per 100 μ L of hydrogel-cell mixture. The ratio between hydrogel-cell mixture and cell culture medium in a well plate is about 1:5 (v/v) (e.g. 600 μ L hydrogel-cell mixture for 3 mL cell culture medium in each well of a 6-well plate).

Optional: Control the final size of the hydrogel-cell beads by adjusting the volume of the droplets. For small beads, 1-5 μ L per droplet and for large beads, 20-50 μ L per droplet).

Tip: Press the pipette plunger to create a droplet on the pipette tip, lower the pipette tip to release the droplet by contacting the surface of culture medium.

6. Place the well plate in an incubator and change the medium every 48-72 hours.

Note: we recommend to only change 50-80% of the top medium without disturbing the hydrogel beads.

PREPARING AN INJECTABLE HYDROGEL

Materials:

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- VitroGel High Concentration hydrogel
- Cells
- Cell culture medium
- VitroGel Dilution Solution (Cat# MS01-100 or MS02-100) or 1X DPBS (no calcium, no magnesium)
- Micropipette; Low retention pipette tips
- Syringe

PROTOCOL

1. Bring VitroGel to room temperature and warm cell culture medium to 37° C if needed.
2. Prepare cell suspension or drug solution in cell culture medium (can also use 1X PBS or VitroGel Dilution Solution to substitute the cell culture medium).
3. Gently mix VitroGel with cell suspension (drug solution) without introducing bubbles. The recommended mixing ratio is listed in Table 5.1 below.

Table 5.1: Volumes of solution/medium for an injectable hydrogel preparation

Medium used to prepare cell suspension/drug solution	VitroGel	Cell suspension/drug solution
Cell culture medium	2 mL	500 µL
1X PBS	1 mL	0.5 - 1 mL
VitroGel Dilution Solution	1 mL	1 mL

4. The mixture can be transferred to a syringe. The hydrogel is ready for injection after stabilization for 10-20 min.

Note:

- The cell concentration needs to be optimized for different applications. We recommend preparing cell suspension at high concentration (10⁶ cells/mL) for the final cell concentration above 2 x 10⁵ cells/mL after mixing with hydrogel solution.
- For different hydrogel concentrations, see Table 5.2 below to prepare injectable hydrogel. We recommend using higher hydrogel concentrations (1:0 to 1:2 (v/v) dilution) for injection applications.

Table 5.2: Volumes of solution/medium for injectable hydrogel preparation

Type of medium to prepare cell suspension/ drug solution	Dilution Ratio	VitroGel	Dilution Solution suspension/ drug solution	Cell Suspension/ Drug Solution	Waiting time for hydrogel stabilization
Cell culture medium	1:0	2 mL	0 mL	500 µL	10-20 min
	1:1	2 mL	2 mL	1 mL	
	1:2	2 mL	4 mL	1.5 mL	
	1:3	1 mL	3 mL	1 mL	
1X PBS	1:0	2 mL	0 mL	1-2 mL	15-30 min
	1:1	2 mL	2 mL	2-4 mL	
	1:2	2 mL	4 mL	2-4 mL	
	1:3	1 mL	3 mL	2-4 mL	

3D CELL CULTURE & 2D COATING HELP GUIDE

- The final cell concentration can be optimized based on different cell types. We recommend to prepare cell suspension at the following concentration for:
3D cell culture: $0.5\text{-}2 \times 10^6$ cells/mL
2D hydrogel coating: $1\text{-}5 \times 10^5$ cells/mL
- After mixing with cell culture medium, immediately transfer the mixture to the tissue culture plate.
Note: If you have multiple samples with different hydrogel conditions or cell types to prepare, transferring mixture of sample 1 to the tissue culture plate before mixing the hydrogel with cell culture medium for sample 2.
- Adding the hydrogel as a dome instead of covering the whole bottom of the well plate might cause hydrogel detachment. Please make sure the hydrogel covers the whole bottom of the well plate.
- During the waiting time (step 4), please do not disrupt the hydrogel by tilting or shaking the well plate.
- Do not over-pipette the hydrogel-medium mixture during the mixing and transferring steps. It might disrupt the hydrogel formation. Pipetting up and down for 5-10 times is sufficient.
- After the initial soft hydrogel formation (step 5), it is important to make sure the hydrogel is stable and attached to the bottom of the well plate before adding the cover medium (if the hydrogel is not stable, it might detached from the bottom of the well after adding the cover medium). ***The gel might be soft, do not shake the plate or align the plate vertically.***

PREPARING CELL SUSPENSION WITH REQUIRED FBS GROWTH FACTORS.

- If cells cultured in complete cell culture medium with 10% FBS or other critical growth factors/supplement, prepare the cell suspension by following the step below:
 - a. Prepare 100% FBS with 10X of critical growth factors.
 - b. Prepare cells in regular 1X cell culture medium (Don't make the medium at higher concentration as the ionic molecules would affect the hydrogel formation).
 - c. Mix the solution a) and b) above to get cell suspension in 50% FBS with 5X critical growth factors.
 - d. Mix the diluted VitroGel with cell suspension at 4:1 v/v ratio (eg. 400 μ L diluted VitroGel with 100 μ L cell suspension).

Note: if the cells need to culture at a higher FBS concentration (eg. 20%), prepare cells suspension directly in 100% FBS. Prepare the diluted VitroGel by mixing VitroGel with VitroGel Dilution Solution and wait 30-60 min before mixing it with cell suspension. Wait 20-30 min at room temperature (or 37°C) before adding the cover medium on top.

PARTIALLY CHANGING THE COVER MEDIUM

- Changing 100% of the cover medium might cause the disruption of the hydrogel by accident. We recommend adding additional fresh medium without removing the top medium at first-time medium change and then only change 60-80% of the cover medium afterward.
(See the recommend volume of the additional cover medium in the Table 6.1 below.)

Table 6.1: Recommend volume for partially cover medium change

	Volume of the cover medium at Day 0	Additional medium volume to add without removing the cover medium at Day 2	Volume of partial medium to change afterwards (Day3)
6 well plate	1200 µL	600 µL	1200 µL
12 well plate	600 µL	300 µL	600 µL
24 well plate	300 µL	150 µL	300 µL
48 well plate	150 µL	50 µL	150 µL
96 well plate	75 µL	25 µL	75 µL

INCREASE HYDROGEL ATTACHMENT BY PRE-COATING THE WELL PLATE

- Using a low VitroGel concentration would make a hydrogel with low elastic modulus. Such hydrogel might detach from the well plate. Pre-coat the well plate with 1X PBS or 10-100 mM CaCl₂ can improve the hydrogel attachment at the bottom of the well plate:
 - Add the PBS or CaCl₂ solution to the well plate for 30 min.
 - Remove the PBS or CaCl₂ solution, open the lid under the biosafety hood for 10-20 min before adding the hydrogel. The recommend volumes of PBS or CaCl₂ for different sizes of well plates are listed in Table 6.2 below.

Table 6.2: Recommend volume of PBS or CaCl₂ solution for pre-coating well plate

	Volume of PBS or CaCl ₂ solution for each well
6-well plate	2000 µL
12-well plate	1000 µL
24-well plate	500 µL
48-well plate	250 µL
96-well plate	100 µL