# 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. 4.1 April 2022

*Check our website for latest protocol guideline revision*

**For Research Use Only. Not for use in diagnostic procedures.**

Information in this document is subject to change without notice.



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:

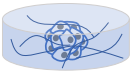
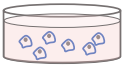
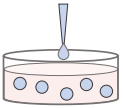
- TYPE 1: Contains sucrose to maintain the best osmolarity.
- TYPE 2: Sucrose free formulation for cells sensitive to the sugar level in the medium.

| 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.<br>**DO NOT FREEZE.** |
| **For research use only. Not for use in diagnostic procedures.** | |

## MULTIPLE CELL CULTURE METHODS WITH VITROGEL HYDROGELS

The XENO-FREE bio-functional VitroGel hydrogel system is versatile for many applications. Choose either the "ready-to-use" hydrogel system for optimized formulation and 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 many research needs. To show the flexibility of our hydrogels, we list five of our most popular cell culture methods that can be performed with our hydrogel: 3D cell culture, 2D hydrogel coating, static suspension culture, hydrogel-cell bead, and as an 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 | 2D HYDROGEL COATING | STATIC SUSPENSION CULTURE | HYDROGEL-CELL BEAD | ANIMAL INJECTION |
|---|---|---|---|---|
| Encapsulate cells in the hydrogel matrix to promote cell-matrix and cell-cell interactions. | Control the substance properties for culturing cells on top of the hydrogel. Great for cell submergence or invasion studies. | Change the viscosity of the cell medium to create a cell suspension environment for large-scale cell expansion. | Encapsulate cells in the hydrogel matrix by creating hydrogel-cell droplets. | 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:
https://www.thewellbio.com/3d-cell-culture-hydrogel/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 rentention 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 0.5-2 x $10^6$ 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 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 |

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 µL diluted VitroGel with 100 µL 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 (µL) | Volume of Cover Medium (µL) |
|---|---|---|
| 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 (µL) | Volume of Cover Medium (µL) |
|---|---|---|
| 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 3 or Table 4 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:

Video Protocol Online

thewellbio.com/video-protocols

- **VitroGel High Concentration hydrogel**
- **Cells**
- **Cell Culture medium**
- **VitroGel Dilution Solution (CAT# MS01-50 or MS02-50)**
- **Micropipette; Low rentention 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 transfering 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 µL diluted VitroGel with 100 µL 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 (µL) | Volume of cells (µL) |
|---|---|---|
| 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 (µL) | Volume of cells (µL) |
|---|---|---|
| 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 x $10^5$ cells/mL.

**Optional Seeding Method:** To ensure cells are seeded on the surface of hydrogel, add 50% of the medium (without cells) on top of hydrogel first. Wait 5-10 min then add the rest 50% of medium with cells on top of the hydrogel. *Example:* For a 24 well plate, add 150 µl medium (without cells) first. Wait 10-15 min. Then, add 150 µl medium with 1-2 x$10^6$ cells/mL on top.

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:

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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 $10^6$ 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

thewellbio.com/video-protocols

- 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 $10^6$ 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 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 µL | 1500 µL | 750 µL | 300 µL | 10 µL |

5. Using a pipettor with a 100 µL tip, carefully pipette the hydrogel-cell mixture from step 3 into the well plate as droplets. (roughly 5-10 droplets per 100 µL 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 µL 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 µL per droplet and for large beads, 20-50 µL 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 of choice**
- **Cells**
- **Cell culture medium**
- **VitroGel Dilution Solution (Cat# MS01-50 or MS02-50) or 1X DPBS (no calcium, no magnesium)**
- **Micropipette; Low rentention pipette tips**
- **Syringe**

Our xeno-free functional hydrogel system is designed based on the application needs and biochemical/biophysical properties. Scientists can choose the hydrogels that fit their research objectives. VitroGel has a unique rheological property that can maintain an injectable status for hours after mixing with cells.

All VitroGel hydrogels are injectable and excellent for xenografts. Researchers have full control of the supplement/growth factors in the hydrogel-cell mixture. Simply adding 3-5X of FBS (or your key supplement) or 3-5% BSA/HSA to the cell suspension before mixing with hydrogel can boost the cell growth after injection.

## 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).**

   **Note:** Adding 3-5X of FBS (or your key supplement) or 3-5% BSA/HSA to the cell suspension before mixing with hydrogel can boost the cell growth after injection.

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. **Transfer the mixture to a syringe. The hydrogel is ready for injection after incubation/stabilization for 10-20 min.**
   *VitroGel has a unique rheological property that can maintain an excellent injectable status for hours.*

## Notes:

- Adding 3-5X of FBS (or your key supplement) or 3-5% BSA/HSA to the cell suspension before mixing with hydrogel can boost the cell growth after injection.
- The cell concentration needs to be optimize for different application. We recommend preparing cell suspension at high concentration ($10^6$ cells/mL) for the final cell concentration above 2 x $10^5$ cells/ml after mixing with hydrogel solution.
- For different hydrogel concentration need, see Table 5.2 below to prepare injectable hydrogel. We recommend using higher hydrogel concentration (1:0 to 1:2 (v/v) dilution) for injection applicatio**n.**

**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 Suspention/ 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 followingconcentration for:
  3D cell culture: 0.5-2 x $10^6$ cells/mL
  2D hydrogel coating: 1-5 x $10^5$ 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 µL diluted VitroGel with 100 µL 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 | Addtional 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 $CaCl_2$ can improve the hydrogel attachment at the bottom of the well plate:
  I. Add the PBS or $CaCl_2$ solution to the well plate for 30 min.
  II. Remove the PBS or $CaCl_2$ solution, open the lid under the biosafety hood for 10-20 min before adding the hydrogel. The recommend volumes of PBS or $CaCl_2$ for different sizes of well plates are listed in Table 6 .2 below.

**Table 6.2: Recommend volume of PBS or $CaCl_2$ 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 |