TheWell BIOSCIENCE

3D Cell Culture and Beyond

VitroGel® Hydrogel Matrix

Catalog #: VHM01S, VHM01
Protocol

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For research use only. Not intended for use in diagnostics or therapeutic procedures.

VitroGel® Hydrogel Matrix

Catalog #: VHM01S, VHM01

INTRODUCTION

VitroGel® Hydrogel Matrix is a ready-to-use, xeno-free (animal origin-free) functional hydrogel for 3D cell culture research. VitroGel® Hydrogel Matrix is an optimized formulation of multi-functional ligands and concentrations to support a wide range of cell types for different applications.

VitroGel® Hydrogel Matrix is ready to use at room temperature and has a neutral pH, transparent, permeable, and compatible with different imaging systems. The solution transforms into a hydrogel matrix by simply mixing with the cell culture medium. VitroGel® Hydrogel Matrix hydrogel is suitable for multiple culture methods including 2D hydrogel coating, 3D cell encapsulation, co-culture, animal injection, and invasion assay. For cell cultured with VitroGel®, the VitroPrime™ Spread-Attach Cell Culture Plate offers an excellent match for superior hydrogel spreading, adherence, and uniform surface to eliminate the edge effect, solve the gel floating issue and uneven cell attachment to help promote rapid cell growth and improve cell yields.

Cells cultured in VitroGel® system can be easily harvested out with our enzyme-free VitroGel® Organoid Recovery Solution (Catalog #: MS04-100 and MS04-500).

IMPORTANT TIPS FOR SUCCESS

- **Before your start,** please visit the following webpage to understand how the gelation process works: https://www.thewellbio.com/3d-cell-culture-hydrogel/how-hydrogel-gelation-works.

- VitroGel® Hydrogel Matrix system is growth factor-free. Before mixing with hydrogel solution, we recommend preparing the cell suspension in a **complete medium with 3X of key supplement** concentration (such as key growth factors, small molecules, FBS, etc.). By using our standard mixing ratio at 2:1, the initial supplement concentration in cell suspension will be reduced to 1X final concentration (1/3 after the mixing). Preparing the cell suspension with 3X of supplements will ensure cells adapt to the new microenvironment smoothly. On the other hand, the medium used to cover on top of the hydrogel (cover medium) or refresh the cover medium can be a regular complete medium with 1X supplement concentration.

- The standard mixing ratio is 2:1 (hydrogel: cell suspension), but if using a cell culture medium with low salt concentration (e.g. RPMI), adjusting the mixing ratio from 2:1 to 1:1 (for ready-to-use hydrogel) can help to accelerate the soft-hydrogel formation.

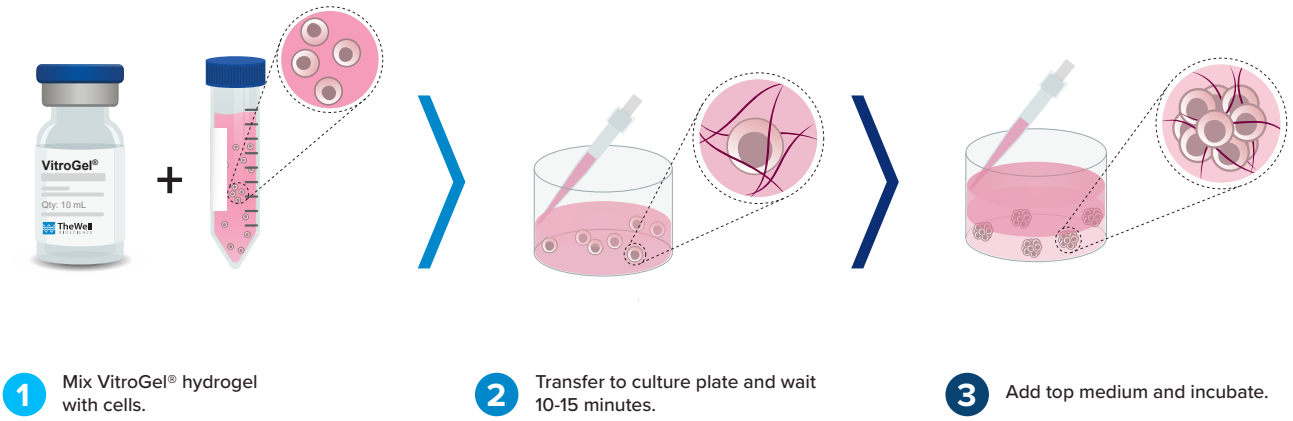
- **The impact of temperature of the gelation process:**
  - » Before mixing with the cell culture medium, adjusting the temperature will only change the viscosity of the hydrogel solution but will not trigger the hydrogel crosslink. At this point, warming up the hydrogel solution will reduce its viscosity.
  - » After mixing hydrogel solution with cell culture medium to trigger the gelation process, **the gelation is faster at a lower temperature and slower at a warm temperature.** Therefore, to ensure a smooth hydrogel formation, incubate soft hydrogel at room temperature for 10-20 min (recommended). If a shorter gelation time is needed, incubate the soft hydrogel at 4°C. If the mixture is incubated at 37°C, it may take a much longer time for the soft hydrogel to form.

Hydrogel preparation for injection: VitroGel® Hydrogel Matrix is ready for injection after mixing with cells for the soft hydrogel formation. To accelerate the soft hydrogel formation and ensure the homogeneous cell suspension within the hydrogel matrix, the hydrogel-cell mixture can be incubated at 4°C or in an ice bucket for 5-10 min for a higher viscosity. The soft VitroGel® performs a unique shear-thinning and rapid recovery rheological property, leading to a smooth injection process and high cell retention rate without clogging the needle or causing the leaking. (To learn more about the injectable properties of VitroGel® system, please visit: https://www.thewellbio.com/3d-cell-culture-hydrogel/unique-injectable-properties-of-vitrogel/; for more information about VitroGel® animal injection applications, please visit: https://www.thewellbio.com/applications/in-vivo/xenograft-pdx-cdx/)

For more information about sample preparation of VitroGel® for cell culture and animal injection, please visit our FAQ page (https://www.thewellbio.com/docs/)

- **Hydrogel/Cell Preparation FAQ:**
  https://www.thewellbio.com/docs-category/hydrogel-cell-preparation/
- **Injectable hydrogel/*in vivo* FAQ:**
  https://www.thewellbio.com/docs-category/injectable-hydrogel-in-vivo/

3D Cell Culture



MATERIALS

- **VitroGel® Hydrogel Matrix (Catalog #: VHM01)**
- Cells
- Cell culture medium
- Additional supplement
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- VitroPrime™ Spread-Attach Plates (Catalog #: VP-SA)

PROTOCOL

1. Bring VitroGel® Hydrogel Matrix to room temperature or warm at 37°C.
2. Prepare cell suspension in the cell culture medium.
   - » Recommended cell concentration 1-2 x 10^6 cells/mL.
   - » **Highly recommended:** To control the critical growth factors/inhibitors/serum in hydrogel, **add desired supplement in cell suspension at 3X concentration.** The cell suspension will then be mixed with VitroGel® Hydrogel Matrix solution to get 1X final concentration in step 3.
3. Add 1 mL VitroGel® Hydrogel Matrix solution to 500 µL cell suspension from step 2 and gently pipette up and down 5-10 times to mix thoroughly. **(Keep VitroGel® and cell suspension at 2:1 v/v mixing ratio)**.

   **If using cell culture medium with low salt concentration such as RMPI 1640 medium, consider using 1:1 v/v mixing ratio. Example, 500 µL VitroGel® hydrogel to 500 µL cell suspension.*

4. Transfer the hydrogel mixture to a well plate. Gently tilt/swirl the well plate to ensure there is an even covering on the bottom of each well. The recommended volumes of hydrogel mixture 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	1200 µL	600 µL	300 µL	150 µL	50 µL

5. Wait 10-15 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.

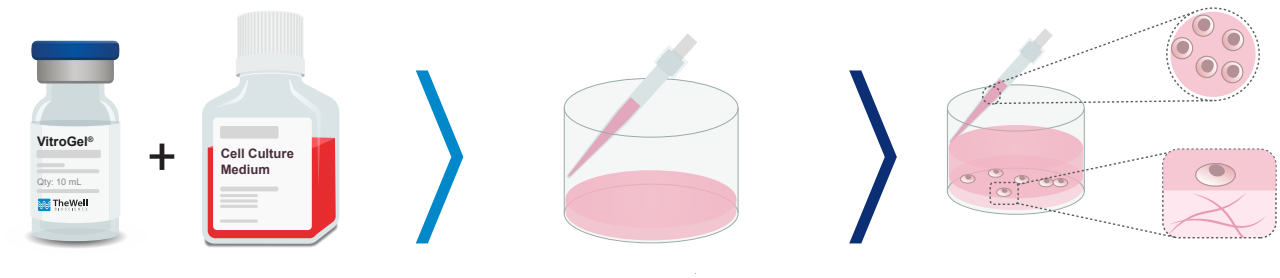
6. Carefully add additional medium (1X medium) to cover the hydrogel. The recommended volumes of cover medium for specific well plate types are listed below.

	6-well plate	12-well plate	24-well plate	48-well plate	96-well plate
Volume per well	1200 µL	600 µL	300 µL	150 µL	50 µL

7. Place the well plate in an incubator. For long term culture, change the cover medium every 48 hours.

   Note: We recommend only changing 50-80% of the top medium without disturbing the hydrogel.

2D Hydrogel Coating



1. Mix VitroGel® hydrogel with culture medium.
2. Transfer to culture plate and wait 10-15 minutes.
3. Add cells on top and incubate.

MATERIALS

- **VitroGel® Hydrogel Matrix (Catalog #: VHM01)**
- Cells
- Cell culture medium
- Additional supplement
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- VitroPrime™ Spread-Attach Plates (Catalog #: VP-SA)

PROTOCOL

1. Bring VitroGel® Hydrogel Matrix to room temperature or warm at 37°C.
2. Add 1 mL VitroGel® Hydrogel Matrix solution to 500 µL cell culture medium and gently pipette up and down 5-10 times to mix thoroughly. (Keep VitroGel® and cell medium at 2:1 v/v mixing ratio).

   ** If using cell culture medium with low salt concentration such as RMPI 1640 medium, consider using 1:1 v/v mixing ratio. Example, 500 µL VitroGel® hydrogel to 500 µL cell medium.*

   » **Highly recommended:** To control the critical growth factors/inhibitors/serum in hydrogel, **add desired supplement in cell culture medium at 3X concentration.** The medium then can mix with VitroGel® hydrogel solution to get 1X final concentration.

3. Transfer the hydrogel mixture to a well plate. Gently tilt/swirl the well plate to ensure there is an even covering on the bottom of each well. The recommended volumes of hydrogel mixture 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	1200 µL	600 µL	300 µL	150 µL	50 µL

4. Wait 10-15 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. At this point, the gelation is faster at a lower temperature and slower at a warm temperature. Therefore, we recommend incubating the soft hydrogel at room temperature to ensure smooth hydrogel formation. If a shorter gelation time is needed, incubate the soft hydrogel at 4°C. If the mixture is incubated at 37°C, it may take a much longer time for the soft hydrogel to form.

5. Carefully add medium with cells on top of hydrogel (Recommend cell concentration of 0.5-1 x 10^6 cells/mL). 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	1200 µL	600 µL	300 µL	150 µL	50 µL

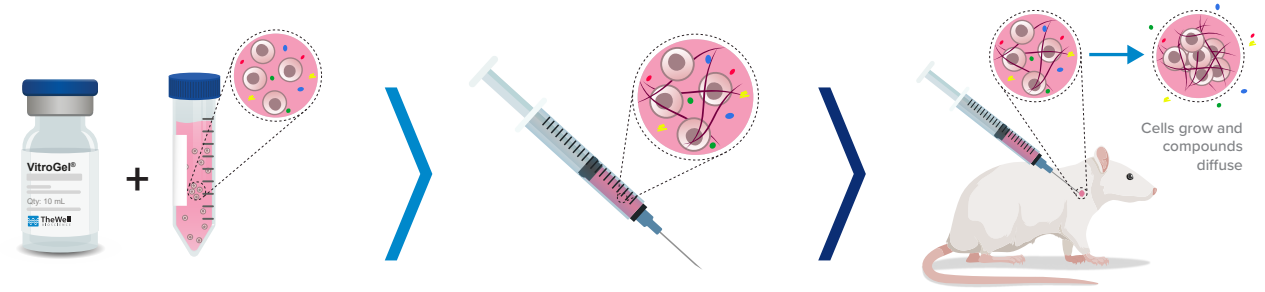
   » Optional Seeding Method: To ensure cells are seeded on the surface of hydrogel, add 50% of the medium (without cells) on top of hydrogel first. Wait 5-10 min for the surface of the hydrogel to solidify, then add the rest 50% of the medium with cells (2X of the desired cell concentration) on top of 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. For long term culture, change the cover medium every 48 hours.

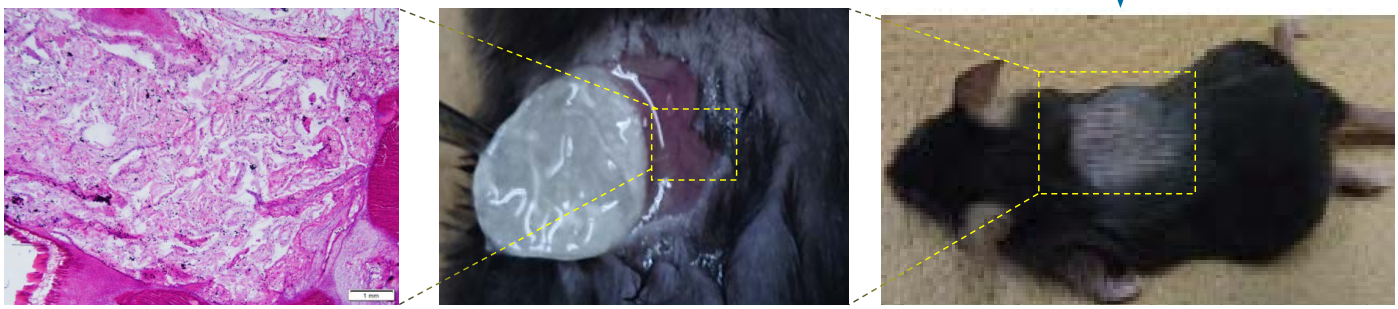
   Note: We recommend only changing 50-80% of the top medium without disturbing the hydrogel.

ANIMAL INJECTION



1. Mix VitroGel® with cell suspension at room temperature.
2. Transfer to syringe & incubate at room temperature for 15 minutes.
3. The stable hydrogel-cell mixture is ready for injection.

Data Example



Hydrogel is stable after injection. The material is biocompatible without toxicity.

MATERIALS

- **VitroGel® Hydrogel Matrix (Catalog #: VHM01)**
- Cells or molecular compounds
- Cell culture medium or PBS
- Additional supplement (optional)
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Centrifuge
- Syringe

PROTOCOL

1. Bring VitroGel® hydrogel to room temperature or warm at 37°C.
2. Prepare cell suspension in PBS.
   - » Adjust the cell/molecular concentration accordingly to the experiment (prepare cell suspension at **2X** desired concentration: the mixing ratio with VitroGel® is 1:1 for **1X** final concentration).
   - » Additional supplement can be added to cell suspension to enhance cell growth after injection
3. Mix VitroGel® hydrogel with cell suspension at 1:1 (v/v) ratio and gently pipette up and down 5-10 times to mix thoroughly.

   Example: 1 mL VitroGel® hydrogel solution to 1 mL cell suspension in PBS.

   (The recommended mixing ratios with other solutions are listed in the table below.)

Medium used to prepare cell suspension/drug solution	VitroGel®	Cell suspension/drug solution
1X PBS at 1:1 gel/cell ratio (v/v)	1 mL	1 mL
Cell culture medium at 2:1 gel/cell ratio (v/v)	2 mL	1 mL

4. Transfer the hydrogel mixture to a syringe. Stabilize the hydrogel mixture either by putting on ice or at 4°C for 5-10 min. Alternatively, stabilize at room temperature for 15 min.
5. After stabilization, the hydrogel mixture is ready for injection. The hydrogel mixture can be kept at room temperature during injections. VitroGel® has a unique rheological property that can maintain an injectable status for hours after mixing with cells without issues of needle clogging.

INVASION ASSAY

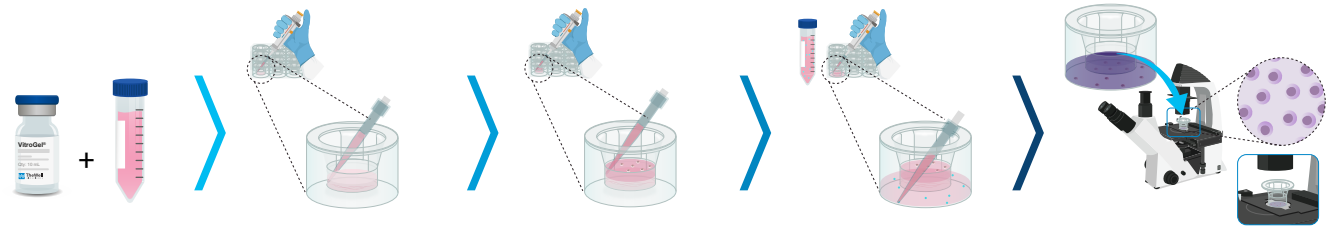
VitroGel® Hydrogel Matrix is ideal for assaying cell invasion and migration. Cell invasion and migration are essential for processes like tissue development, immune responses, wound healing, and cancer spread. During invasion, cells undergo morphological changes, degrade the extracellular matrix, and respond to environmental signals.

Our advanced VitroGel® hydrogel system closely replicates the native ECM's physical and functional properties, offering a perfect balance of biological complexity and ease of use.

Different invasion assays can be performed with VitroGel® Hydrogel Matrix:

- Vertical invasion assays
- Spheroid invasion assays
- Horizontal invasion assays

Vertical invasion assay



1. Mix VitroGel® hydrogel with cell medium at a 2:1 ratio.
2. Seed hydrogel mixture to the insert. Wait for 20 min.
3. Seed 100 µL of the cell suspension on top of the insert.
4. Pipette 500 µL of medium + factors of interest to the outer well
5. Evaluate invasion/migration using crystal violet staining.

MATERIALS

- **VitroGel® Cell Invasion Assay Kit (Catalog #: IA-VHM01), which includes:**
  - **VitroGel® Hydrogel Matrix (Catalog #: VHM01)**
  - **VitroPrime™ Cell Culture Inserts (8 µm) in a 24-well plate (Catalog #: VPE8-24)**
- Cells
- Cell culture medium
- Additional supplement (optional)
- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips

PROTOCOL

1. Allow VitroGel® Hydrogel Matrix and culture medium to reach room temperature.
2. Add 1 mL VitroGel® Hydrogel Matrix to 500 µL basal cell culture medium and gently pipette 5-10 times to homogenize the mixture. (Keep VitroGel® hydrogel solution and basal cell culture medium at 2:1 v/v mixing ratio).

   **If using cell culture medium with low salt concentration, such as RMPI 1640 medium, consider using 1:1 v/v mixing ratio (Example, 500 µL VitroGel® hydrogel solution to 500 µL cell culture medium).*

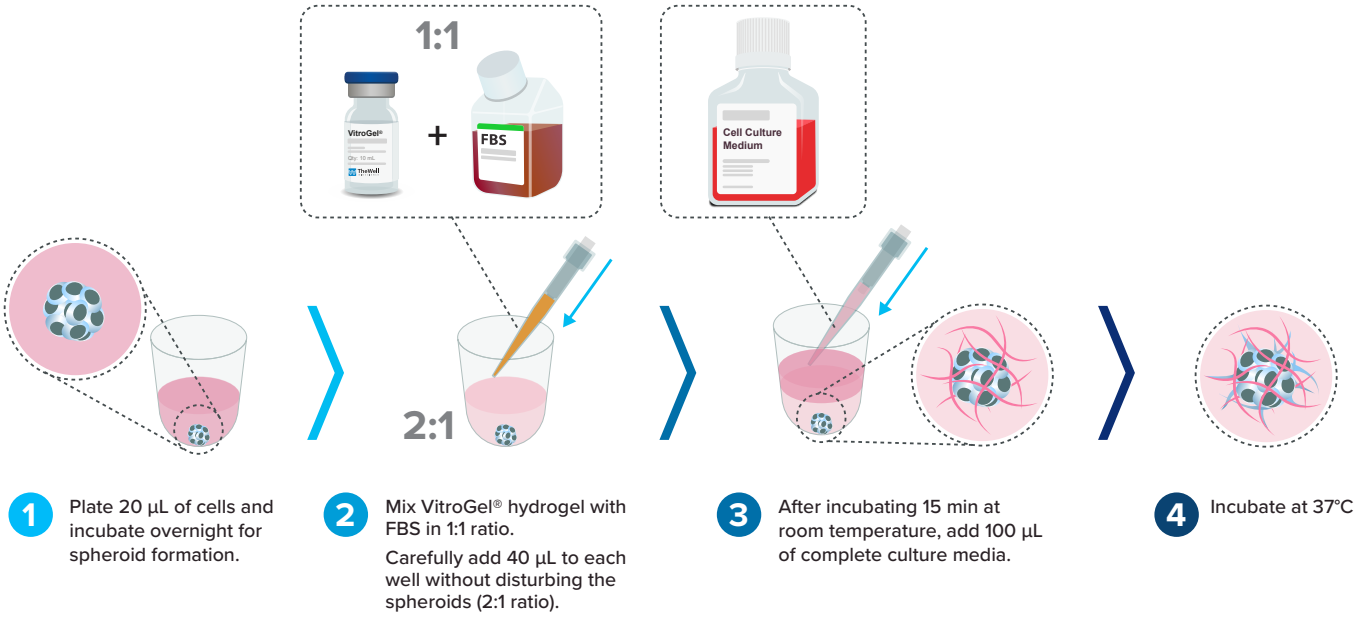
   Note: If you need to add supplements such as cytokines, growth factors, chemokines, or chemical agents to the hydrogel matrix, add the 3X desired concentrations of supplement to the cell culture medium. The cell culture medium then can mix with VitroGel® hydrogel solution to get 1X final supplement concentration in hydrogel matrix. (Examples, Prepare medium with 30 ng/mL of the cytokine. Mix VitroGel® hydrogel solution with the medium at a 2:1 v/v mixing ratio to obtain a final concentration of 10 ng/mL cytokine inside the hydrogel matrix).
3. Add 100 µL of the hydrogel mixture to each insert and ensure there is an even hydrogel covering on the surface of each insert.
4. Allow hydrogel mixture to solidify for 20 minutes at room temperature before adding the cells on top of the hydrogel.
5. Prepare cell suspension in the desired culture medium (i.e., serum-free medium) at the concentration of 1-3 x 10^5 cells/mL and add 100 µL of cell suspension on top of the hydrogel.

   Optional Seeding Method: to ensure cells are seeded on the surface of the hydrogel, add 50% of the medium (without cells) on top of the hydrogel first. Wait 5-10 min then add the rest 50% of the medium with cells on top of the hydrogel. (For example, add 50 µL medium (without cells) first; wait 10-5 min; then add 50 µL medium with 2-6 x 10^5 cells/mL on top).
6. Prepare cell culture medium with factors of interest (i.e., chemokines, cytokines, or serum), and add 500 µL of cell culture medium to the outer wells.
7. Incubate cells in a humidified cell culture incubator at 37°C.

For more information, please check the following reference:

- **VitroGel® Cell Invasion Assay Kit product page:**
  https://www.thewellbio.com/product/vitrogel-cell-invasion-assay-kit/
- **VitroGel® Cell Invasion Assay Kit protocol:**
  https://www.thewellbio.com/wp-content/uploads/2024/11/Protocol_Cell-Invasion-Assay-Ready-to-Use.pdf
- **Crystal Violet Staining Protocol:**
  https://www.thewellbio.com/wp-content/uploads/2024/11/Protocol_Cell-Invasion-Assay-Ready-to-Use.pdf

Spheroid Invasion Assay



MATERIALS

- **VitroGel® Hydrogel Matrix (Catalog #: VHM01)**
- Cells
- U-shaped bottom and ultra-low attachment 96-well plate
- Complete cell culture medium with supplement
- Cell culture medium supplement (e.g. Fetal Bovine Serum (FBS))
- Micropipette; low retention pipette tips
- Centrifuge tubes or conical tubes
- Parafilm sealant

PROTOCOL

Step I: Spheroid formation

1. Harvest the cells and resuspend a total of 1 x 10^6 cells in 1 mL of complete culture medium.
2. Add 20 µL of the cell suspension to each well of an ultra-low attachment and u-shaped bottom 96-well plate.
3. Place the cells in a humidified cell culture incubator at 37°C overnight.

Step II: Addition of hydrogel to evaluate spheroid invasion

1. Allow VitroGel® Hydrogel Matrix, FBS, and complete cell culture medium to reach room temperature.

2. Combine the hydrogel with FBS in a 1:1 ratio. Homogenize gently 3-5 times using a 1 mL micropipette.
3. Remove the cell culture plate from the incubator and gently add the hydrogel-FBS mixture to the well with the spheroid in a 2:1 ratio (v/v, hydrogel/medium volume). For example, add 40 µL of hydrogel mixture to the wells containing the spheroids cultured in 20 µL of complete cell culture medium.

   Note: To avoid disrupting the spheroid, tilt the plate at a 45° angle and place the pipette against the wall of the well to add the hydrogel.
4. Incubate the hydrogel for 15 minutes at room temperature.
5. Add a 100 µL of complete cell culture medium on top of the hydrogel.

   Note: Place the pipette against the walls of the wells to add the medium.
6. Incubate the spheroids in a humidified cell culture incubator at 37°C.
7. Monitor and assess spheroid invasion using a microscope. Change the medium every 2-3 days.

Cell Harvesting Protocol

MATERIALS

- Cells cultured with VitroGel® system
- **VitroGel® Organoid Recovery Soultion (Catalog #: MS04-100)**
- DPBS (Wash Buffer, no calcium, no magnesium)
- Conical tubes (15 mL or 50 mL)
- Serological pipettes
- Micropipette; Low retention pipette tips
- Dry bath or water bath set to 37°C
- Centrifuge
- Lab Spatula

PROTOCOLS

Using 24 well-plate, 300 µL gel/well as an example

The selection of METHOD 1 and METHOD 2 below depends upon the conditions of cells and hydrogel: If the sizes of cells in hydrogel are bigger than 500 µm in diameter, Method 1 is recommended; if using VitroGel® at a high gel concentration (1-0 or 1-1 dilution) or the sizes of cells in hydrogel are smaller than 500 µm in diameter, Method 2 is recommended.

METHOD 1

Using a serological pipette to break the hydrogel into smaller pieces

1. Warm the VitroGel® Organoid Recovery Solution to 37°C.
2. Take the cells out of the incubator and remove the medium covering the top of the hydrogel. Wash the hydrogel two times with DPBS.
3. Add 1 mL warm VitroGel® Organoid Recovery Solution to the well and use a 10 mL serological pipette to gently break the hydrogel into small pieces by gently pipetting up and down. This step can accelerate the hydrogel dissolving process.
4. Add 5 mL warm VitroGel® Organoid Recovery Solution to a 15 mL conical tube and transfer the hydrogel to the tube. Optional: Rinse the well with 1 mL warm VitroGel® Organoid Recovery Solution and combine the solution to the centrifuge tube.
5. Use a 10 mL serological pipette to gently pipette the mixture up and down 2-5 times and put the tube back to the water bath for 2- 3 min. Repeat this cycle 2-3 times. (Optimize the pipetting times and repeat according to the gel strength and cell type).
6. Centrifuge at 100 x g for 3-5 minutes at room temperature to collect the cell pellet. (Optimize the speed and time of centrifuge according to different cell types).

   Optional: If there is still some hydrogel on top of the cell pellet, resuspend the cell with 5 mL warm VitroGel® Organoid Recovery Solution and repeat steps 4 to 6 one more time.

METHOD 2

Using a lab spatula

1. Warm the VitroGel® Organoid Recovery Solution to 37°C.
2. Take the cells out of the incubator and remove the medium covering the top of the hydrogel. Wash the hydrogel two times with DPBS.
3. Add 1 mL warm VitroGel® Organoid Recovery Solution to the well and use a spatula to detach the hydrogel from the well plate.
4. Add 5 mL warm VitroGel® Organoid Recovery Solution to a 15 mL conical tube and transfer the hydrogel to the tube.

   Optional: Rinse the well with 1 mL warm VitroGel® Organoid Recovery Solution and combine the solution to the centrifuge tube.
5. Rock the conical tube 20 times and then put the tube back in the water bath for 2-3 minutes. Repeat this cycle 3-5 times. (Optimize the rocking time and the repeats according to the gel strength and cell type).
6. Centrifuge at 100 x g for 3-5 minutes at room temperature to collect the cell pellet. (Optimize the speed and time of centrifuge according to different cell types).

   Optional: If there is still some hydrogel on top of the cell pellet, resuspend the cell with 5 mL warm VitroGel® Organoid Recovery Solution and repeat steps 5 and 6 one more time.

IMPORTANT NOTES FOR BOTH METHODS:

- **KEEP THE SOLUTION WARM**: It is important to keep the VitroGel® Organoid Recovery Solution and the mixture warm at 37°C during the whole process. The warm temperature is essential to accelerate molecular exchanges to release the ionic molecules from the solid hydrogel, which can transform into a soft hydrogel.
- **APPLY MECHANICAL FORCE**: The mechanical force such as rocking the tube or using a serological pipette to mix the hydrogel with the VitroGel® Organoid Recovery Solution helps to transform the hydrogel into the liquid state.
- **DILUTION**: Adding the VitroGel® Organoid Recovery Solution at the volume of 10X or higher than the hydrogel maintains the dissolved hydrogel in a liquid state.
- **CENTRIFUGE AT ROOM TEMPERATURE.**

See how cell harvesting works:
https://www.thewellbio.com/3d-cell-culture-hydrogel/cell-harvesting/

VIDEO PROTOCOLS

- **Ready-to-use VitroGel®:** https://www.thewellbio.com/3d-cell-culture-with-VitroGel®-ready-to-use-hydrogels/
- **Cell recovery from 3D cell culture:** https://www.thewellbio.com/3d-2d-cell-recovery/
- **Injectable hydrogel:** https://www.thewellbio.com/xenograft-injection-video

DATA EXAMPLES

- **2D hydrogel coating applications:** https://www.thewellbio.com/applications/2d-cell-culture/
- **Invasion assay applications:** https://www.thewellbio.com/applications/invasion-assay/

DOWNSTREAM ANALYSIS

- **Cell harvesting:** please check protocol on page 14
- **Cell viability:** https://www.thewellbio.com/wp-content/uploads/2020/11/Protocol_Cyto3D-Live-Dead-Assay-Kit.pdf
- **Immunofluorescence Staining:** https://www.thewellbio.com/wp-content/uploads/2020/08/Protocol_Immunofluoroscence-Staining.pdf
- **Sample Preparation for Histological Analysis:** https://cdn.thewellbio.com/wp-content/uploads/2020/08/Protocol_Sample-Preparation-for-Histological-Analysis.pdf
- **Cell Fluorescent Staining in Gel for Nuclei and Actin Filament:** https://cdn.thewellbio.com/wp-content/uploads/2020/08/Protocol_Cell-Fluorescent-Staining-in-Gel-for-Nuclei-and-Actin-Filament.pdf
- **Cell Proliferation Assay:** https://www.thewellbio.com/wp-content/uploads/2020/12/Cell-Proliferation-Assay-Protocol.pdf
- **RNA/DNA Extraction, Cell Lysis Protocol:** https://www.thewellbio.com/wp-content/uploads/2020/08/Protocol_Cell-Lysis-for-Downstream-Analysis.pdf

FAQs

- **General hydrogel questions:** https://www.thewellbio.com/docs-category/general-hydrogel-questions/
- **Hydrogel/cell preparation:** https://www.thewellbio.com/docs-category/hydrogel-cell-preparation/
- **Downstream analysis (Imaging/DNA extraction, etc.):** https://www.thewellbio.com/docs-category/analysis/
- **Injection/*in vivo*:** https://www.thewellbio.com/docs-category/injectable-hydrogel-in-vivo/
- **3D cell models and functional assays:** https://www.thewellbio.com/docs-category/3d-cell-models-functional-assays/
- **Organoids:** https://www.thewellbio.com/docs-category/organoids/

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