PROTOCOL

VitroGel® Organoid Recovery Solution

CAT NO. MS04-10, MS04-100, MS04-500

NON-ENZYMATIC CELL HARVESTING SOLUTION TO RECOVER CELLS / ORGANOIDS FROM HYDROGEL OR AN ANIMAL-BASED ECM WITHIN 15 MINUTES.

VitroGel® Organoid Recovery Solution is an enzyme-free cell harvesting solution for quick and efficient cell recovery from cells or organoids cultured with VitroGel or an animal-based ECM. It is superior to other top leading cell recovery solutions on the market and can recover cells more quickly and efficiently. This solution is enzyme-free and is room temperature stable, with a neutral pH. The solution can maintain high cell viability during the recovery process. Harvested cells can be sub-culture in both 3D and 2D cultures. This solution supports excellent cell recovery for organoid expansion. It can be used before or after the fixation and stained preparation of hydrogel specimens to ensure high-quality downstream data analysis. VitroGel Organoid Recovery Solution replaces the VitroGel Cell Recovery Solution. Users will notice a quicker and more efficient recovery using the VitroGel Organoid Recovery Solution.

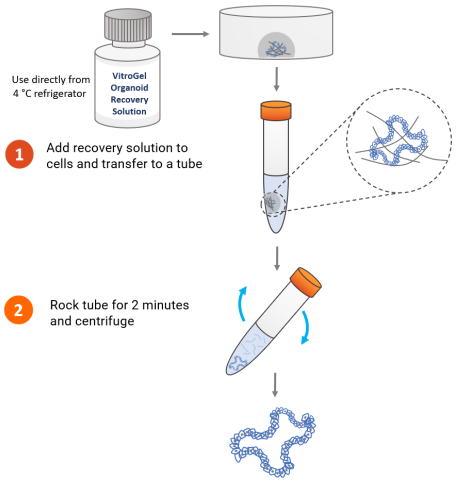
Features and Benefits

- **Harvest Organoid/Cells** – Recovery of intact organoids as well as 3D cells
- **Time Savings** – Fast 2-minute ECM dissociation of animal-based ECM for intact organoids/cells
- **High-Yield** – Complete ECM dissociation for high recovery rate for organoid expansion
- **Downstream** – Recovered cells can be sub-cultured in both 3D and 2D
- **Non-enzymatic** – Enzyme-free for stable, safe and efficient cell recovery
- **Easy-To-Use** – Room temperature operation
- **3D and 2D ECM** – Supports cell recovery from 2D ECM coating plates
- **Stable Formulation** – Stable for 15 months. No cold pack shipping. Store at 2-8°C.

CELL / ORGANOID RECOVERY WORKFLOW

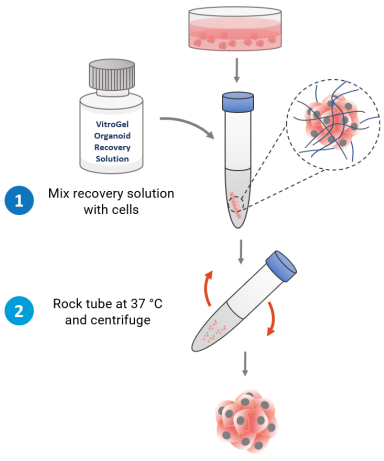
Cell / Organoid recovery from animal-based ECM (e.g. Matrigel)

Less than 10 min protocol. Fast 2 minute ECM dissociation.



Cell / Organoid Recovery from VitroGel Hydrogels

Less than 15 min protocol.



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*Extracellular matrices like Matrigel, Cultrex, and Geltrex

CELL / ORGANOID RECOVERY FROM ANIMAL-BASED ECM*

RECOMMENDED MATERIALS AND REAGENTS

- VitroGel Organoid Recovery Solution (MS04-100 or MS04-500)
  Note: If soley using for cell harvesting from animal-based ECM, store at 4°C until usage.
- 15 mL centrifuge tubes
- Serological pipettes
- Centrifuge capable of 4 °C
- Ice bucket

PROTOCOL: (using a 24 well plate with 25 µL Matrigel dome/well as an example)

BEFORE STARTING

⚠ **For animal-based ECM cell / organoid harvesting, chill VitroGel Organoid Recovery Solution to 4°C before use for optimal performance.**

1. Remove the medium from the animal-based ECM culture plate.
2. Add 1 mL cold VitroGel Organoid Recovery Solution (pre-chilled at 4 °C) to each well of a 24-well plate.
3. Transfer the gel and solution together to a 15 mL centrifuge tube.
   Optional: Add another 1 mL VitroGel Organoid Recovery Solution to wash the plate and combine the solution to the tube.
4. Gently pipette up and down 5-10 times and chill the tube on ice or place in refrigerator for 2-5 minutes for ECM dissociation.

   Optional: Rock the tube for 2-5 minutes instead of pipetting.
5. Centrifuge at 100 x *g* for 3-5 minutes at 4 °C.
6. Remove the supernatant. The cells should be ready for subculture or storage.

   Optional: To break organoids into smaller fragments, resuspend the cell in a culture medium, pipette thoroughly and centrifuge again.

   ***Note: Because sources of animal-based ECM have varying protein concentrations, it may require to re-suspend the cells in additional VitroGel Organoid Recovery Solution (repeat steps 4 to 6) to completely remove the ECM.**

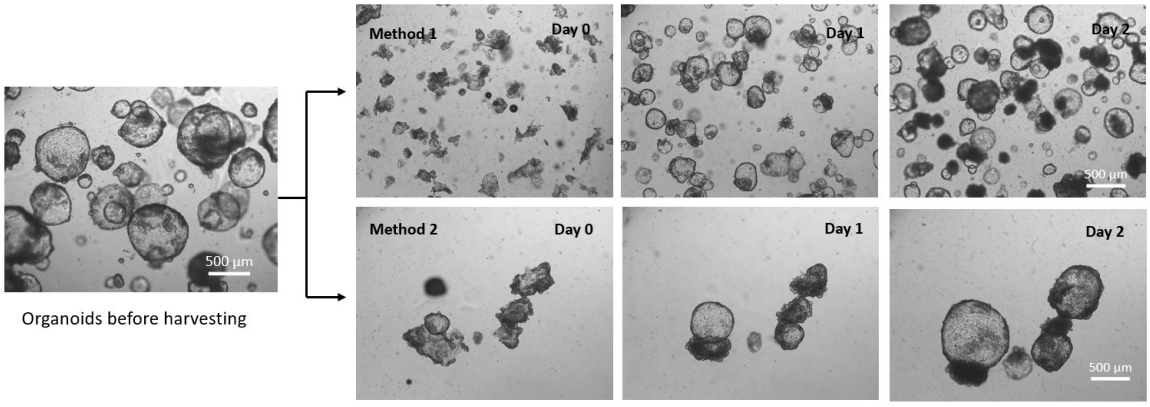

Figure 1. Organoids recovered from Matrigel by using VitroGel Organoid Recovery solution with two different methods.
Method 1: re-suspend organoids in VitroGel Organoid Recovery Solution by pipetting to break organoids into small fragments for sub-culture/expansion.
Method 2: rocking the tube with organoids and VitroGel Organoid Recovery Solution mixture without using a pipette to receive the intact organoids. VitroGel Organoid Recover Solution was kept in a 4°C refrigerator to maintain a low temperature before use. The organoids/Matrigel and VitroGel Organoid Recovery Solution mixture were incubated at room temperature for 2 min before centrifuging. Day 0 images show the morphology of organoids right after harvesting with two different methods.

*Extracellular matrices like Matrigel, Cultrex, and Geltrex

IPSC RECOVERY FROM 2D ANIMAL-BASED ECM* COATING PLATE

RECOMMENDED MATERIALS AND REAGENTS

- VitroGel Organoid Recovery Solution (MS04-100 or MS04-500)
- Cell culture medium
- 15 mL centrifuge tubes
- Serological pipettes
- Incubator (Set to 37 °C)
- Centrifuge capable of 4 °C

PROTOCOLS: (using iPSC cells cultured on 6 well Matrigel coating plate as an example)

1. Remove the medium from the animal-based ECM culture plate.
2. Add 2 mL of PBS solution to wash each well of a 6-well plate.
3. Add 1 mL of the VitroGel Organoid Cell Recovery Solution to each well of a 6-well plate.
4. Incubate the plate at room temperature for 3-5 minutes.
   Optional: Place the plate in refrigerator for 2-5 minutes to accelerate ECM dissociation.
5. Add 1 mL of medium to each well and transfer the cell suspension to a 15 mL centrifuge tube.
6. Wash the plate with 1 mL medium twice and combine the medium to the centrifuge tube.
7. Centrifuge at 100 x *g* for 3-5 minutes at 4 °C.

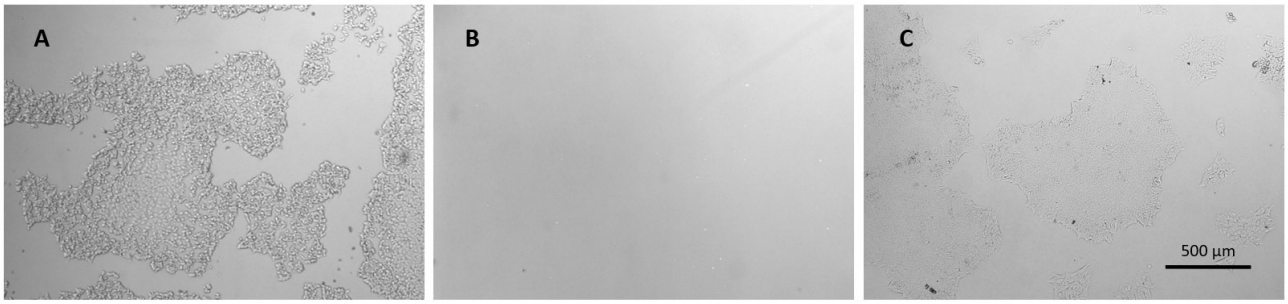

Figure 2. Use VitroGel Organoid Recovery Solution for iPSC harvesting from 2D Matrigel coating plate
VitroGel Organoid Recovery Solution can be used to harvest iPSC cells from a 2D Matrigel coating plate. The solution was warmed up to room temperature before use. A) Morphology of cells detaching from the Matrigel coating plate. (3 min after adding VitroGel Organoid Recovery Solution), B) Image of the well plate after cell harvesting. (Shows all cells were removed from the Matrigel coating plate), C) Morphology of cells after re-seeding to a new Matrigel coating plate (Day 3).

*Extracellular matrices like Matrigel, Cultrex, and Geltrex

CELL / ORGANOID RECOVERY (3D & 2D CULTURE) FROM VITROGEL

RECOMMENDED MATERIALS AND REAGENTS

- Cells cultured with VitroGel system
- VitroGel Organoid Recovery Soultion (Cat# 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 ^{o}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.

ONLINE VIDEO PROTOCOL: **https://www.thewellbio.com/3d-2d-cell-recovery/**

METHOD 1 (using a serological pipette to break the hydrogel into smaller pieces)

1. Warm the VitroGel Organoid Recovery Solution to 37 ^{o}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 tot he water bath for 2- 3 min. Repeat this cycle 2-3 times. (Optimize the pipetting times and repeats according to the gel strength and cell type).
6. Centrifuge at 100 x g for 3-5 minnutes 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 ^{o}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.

CELL / ORGANOID RECOVERY (3D & 2D CULTURE) FROM VITROGEL

METHOD 2 (continued)

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 Organoid Recovery Solution and the mixture warm at 37 ^{o}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 Organoid Recovery Solution helps to transform the hydrogel into the liquid state.
- DILUTION: Adding the 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

CELL / ORGANOID RECOVERY FROM CELL STATIC SUSPENSION CULTURE IN VITROGEL

RECOMMENDED MATERIALS AND REAGENTS

- Cell static suspensional cultured in VitroGel system
- VitroGel Organoid Recovery Soultion (Cat# MS04-100)
- Conical tubes (15 mL or 50 mL)
- Serological pipettes
- Micropipette; Low retention pipette tips
- Dry bath or water bath set to 37 ^{o}C
- Centrifuge

PROTOCOLS: (using 6 well-plate, 3 mL cell suspension per well as an example)

1. Warm the VitroGel Organoid Recovery Solution to 37 ^{o}C.
2. Take the cells out of the incubator and transfer the cell suspension to a 15 mL conical tube by using a 10 mL serological pipette.
3. Add additional 5 mL warm VitroGel Organoid Recovery Solution to a 15 mL conical tube.
4. Use a 10 mL serological pipette to gently pipette the mixture up and down 3-5 times and put the tube to a dry bath or water bath and incubate at 37°C for 3-5 minutes.

   Optional:

   Pipette the mixture up and down 3-5 times again before centrifuge.
5. 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 with 5 mL warm Organoid Recovery Solution and repeat steps 4 and 5 one more time.

IMPORTANT NOTES:

- KEEP THE SOLUTION WARM: It is important to keep the VitroGel Organoid Recovery Solution and the mixture warm at 37 ^{o}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

CELL / ORGANOID RECOVERY FROM HYDROGEL-CELL BEAD CULTURE IN VITROGEL

RECOMMENDED MATERIALS AND REAGENTS

- Cells cultured in VitroGel-cell beads
- VitroGel Organoid Recovery Soultion (Cat# MS04-100)
- Conical tubes (15 mL or 50 mL)
- Serological pipettes
- Micropipette; Low retention pipette tips
- Dry bath or water bath set to 37 °C
- Centrifuge

PROTOCOLS:

The ratio between the hydrogel-cell beads (no medium) and VitroGel Cell Recovery Solution is approximately 1:10 v/v.
Example below: Using a 6 well-plate with 600 µL hydrogel-cell beads in 3 mL medium per well, 5 mL VitroGel Cell Recovery Solution is used.

ONLINE VIDEO PROTOCOL: **https://www.thewellbio.com/msc-hydrogel-bead-cell-harvesting**

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 carefully remove the cell culture medium from the well without disrupting the hydrogel-cell beads.
3. Add 5 mL warm VitroGel Organoid Recovery Solution to the well and transfer the hydrogel-cell beads to a 15 mL conical tube by using a 10 mL serological pipette. (The ratio between hydrogel-cell beads and Organoid Recovery Solution is approximately 1:10 v/v.)
4. Using the serological pipette, gently pipette the mixture up and down 3-5 times and put the tube to a dry bath or water bath and incubate at 37°C for 3-5 minutes.

   Optional:

   Pipette the mixture up and down 3-5 times again before centrifuge.
5. 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 with 5 mL warm Organoid Recovery Solution and repeat steps 4 and 5 one more time.

IMPORTANT NOTES:

- KEEP THE SOLUTION WARM: It is important to keep the Organoid Recovery Solution and the mixture warm at 37 ^{o}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 Organoid Recovery Solution helps to transform the hydrogel into the liquid state.
- DILUTION: Adding the 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