IMMUNOLOGICAL SCIENCES Product Data Sheet

MATRIXGEL
Product Experimental Manual

Cat.n.	Product Name	Size
MX0314-5	MatrixGel for Angiogenesis, Invasion, Tumorigenesis, **Containing Phenol Red**	5 ml
MX0314-10		10 ml
MX1314-5	MatrixGel For Angiogenesis, Invasion, Tumorigenesis, **Phenol Red-Free**	5 ml
MX1314-10		10 ml
MX2314-5	MatrixGel For Organoids, 3D Culture, **Containing Phenol Red**	5 ml
MX2314-10		10 ml
MX3314-5	MatrixGel For Organoids, 3D Culture, **Phenol Red-Free**	5 ml
MX3314-10		10 ml

1.2Product Description

At room temperature, MatrixGel can quickly polymerize to form a biologically active three-dimensional matrix, mimicking the composition, structure, physical properties, and functions of the basement membrane of cells in vivo. It can promote the proliferation and differentiation of various cells in vitro, such as epithelial cells, endothelial cells, melanoma cells, and stem cells. It plays a crucial role in studying cell morphology, physiological functions, invasion, and promoting tumorigenesis in difficult-to-tumorigenic cells. MatrixGel is a sterile product and is free of viruses that affect experimental animals. With a protein concentration of 8-12 mg/mL, it can be used in experimental studies such as angiogenesis, in vivo tumorigenesis, 3D organoid culture, and tumor cell invasion.

1.3Storage and Transportation

Dry ice transportation: Store in darkness at -20°C with a validity period of 2 years. After initial thawing, please properly aliquot and store in a -20°C freezer. Do not store in a frost-free refrigerator.

1.4Product Characteristics

(1) MatrixGel may undergo color changes during freezing and thawing due to the interaction between the bicarbonate buffer in the product, carbon dioxide, and phenol red. The color may change from pale yellow to deep red, which will disappear under 5% CO_2 equilibrium and is a normal phenomenon that does not affect product functionality. The phenol red-free version has a white or pale yellow color and does not exhibit this phenomenon.

(2) MatrixGel is prone to gelation, so it should be placed in ice and stored overnight in a 4°C refrigerator. Once thawed, the vial can be vortexed to ensure product uniformity, and it should not be vigorously pipetted to avoid generating large bubbles, which may affect product performance.

(3) MatrixGel is a gel-like liquid that gradually gelates above 10°C. Therefore, during use, all items directly in contact with it, such as pipette tips, culture dishes, culture plates, and culture medium, should be pre-cooled or

frozen.

(4) The protein concentration of this product varies between batches, with the specific concentration indicated in the Certificate of Analysis (COA). The amount of MatrixGel to be used should be determined based on experimental requirements and the specific protein concentration to ensure experimental accuracy, but it is not recommended to dilute the product below 3 mg/mL.

2 Methods for Using MatrixGel

2.1 Thawing and Aliquoting of MatrixGel (Keep the product on ice throughout the process)

(1) Thawing: Place the product in ice and store it overnight in a 4°C refrigerator. Avoid placing it on the refrigerator door or in a frequently opened refrigerator to prevent temperature fluctuations from affecting product performance. After thawing, vortex the vial to ensure product uniformity.

(2) Aliquoting: According to experimental needs, use pre-cooled pipette tips or pipettes to aspirate the product and dispense it into pre-cooled sterile centrifuge tubes. Then store the aliquots at -20°C or below in a stable and light-protected environment. If the pipette tip or pipette becomes clogged during aspiration, replace it promptly with a new pre-cooled one, and ensure sterile operation during aliquoting.

2.2 Common Coating Methods for MatrixGel

MatrixGel can be coated in various methods, such as thin gel method, thick gel method, and thin layer coating method, which are suitable for different experiments. The appropriate coating method can be selected according to the specific experimental purposes.

2.2.1 Thin Layer Gel Method

Forms a gel layer of approximately 0.5 mm thickness, on which cells are plated for culture. This method is mainly suitable for cell adhesion and proliferation, such as in angiogenesis experiments.

(1) Thaw MatrixGel the day before by placing it in ice and storing it overnight in a 4°C refrigerator. After thawing, vortex the vial or use pre-cooled pipette tips/pipettes to gently mix MatrixGel until uniform.

(2) Place the culture plate on ice and add MatrixGel at 50 μL/cm² to the growth surface.

(3) Incubate the culture plate at 37°C for 30 minutes to solidify the MatrixGel.

(4) (Optional) Before use, aspirate unbound material using serum-free medium and gently rinse, ensuring that the pipette tip does not scratch the coated surface.

2.2.2 Thick Layer Gel Method

Forms a gel layer of approximately 1-2 mm thickness, with cells growing inside the gel. This method is mainly

suitable for experiments such as 3D organoid culture.

(1)-(4) Follow steps (1)-(4) of the Thin Layer Gel Method, but increase the volume of MatrixGel added to 150-200 μL/cm² and suspend cells in the MatrixGel before adding.

2.2.3 Thin Layer Coating Method

Uses a lower concentration of MatrixGel to form a mixed protein coating layer without gelation, on which cells are plated for culture. This method is mainly suitable for cell adhesion experiments and can also be used for tumor cell Tranll invasion assays in vitro.

(1)-(4) Follow steps (1)-(3) of the Thin Layer Gel Method, but dilute MatrixGel with pre-cooled serum-free medium or PBS to the desired concentration before adding. Incubate at room temperature for 1-2 hours (determined based on MatrixGel solidification time) before aspirating unbound material and gently rinsing with serum-free medium for use.

3 Experimental Methods for Using MatrixGel

MatrixGel can be applied in in vitro angiogenesis experiments, Tranll invasion experiments of tumor cells, tumor formation promotion experiments of difficult-to-tumorigenic cells, organoid experiments, and 3D spheroid experiments, etc.

3.1 In Vitro Angiogenesis Experiment (Taking PUMC-HUVEC-T1 as an Example)

3.1.1 Experimental Materials

MatrixGel (MX0314/MX1314)

PUMC-HUVEC-T1 cells

PUMC-HUVEC-T1 cell-specific medium

Recombinant Trypsin-EDTA Solution without Phenol Red

Calcein AM

96-well plate

200 μL pipette tips

1.5mL centrifuge tubes

Pipettor (SPIP-200)

3.1.2 Experimental Steps

Place the MatrixGel in an ice box and thaw it overnight in a 4°C refrigerator the day before the experiment to allow the MatrixGel to melt slowly. At the same time, pre-cool the 96-well plate, 200 μL pipette tips, and 1.5 mL centrifuge tubes required for the experiment in a 4°C refrigerator.

After thawing, vortex the vial or use a pre-cooled pipette/pipette tip to gently mix the MatrixGel until it is uniform. Ensure that the MatrixGel is always placed on ice during the experiment.

Dilute the MatrixGel to the desired concentration in a 1.5 mL centrifuge tube using the PUMC-HUVEC-T1 cell-specific medium (based on the experiment, the MatrixGel can be diluted 1:1 with the medium for use).

Place the 96-well plate on ice and add 50 μL of MatrixGel per well (a MatrixGel protein concentration of 6-8 mg/mL is recommended for best results, which can be diluted according to the total protein concentration of the MatrixGel before use). During this process, ensure that the pipette tip is perpendicular to the top of the well and add slowly to avoid generating bubbles. If the bottom of the well is not fully covered with MatrixGel, gently shake the 96-well plate to evenly spread the gel on the bottom, and then place the 96-well plate in a 37°C incubator for 45-60 minutes to solidify the MatrixGel.

Digest the pre-recovered and cultured PUMC-HUVEC-T1 cells using recombinant trypsin cell digestion solution. After complete digestion, add the specific medium to terminate the digestion. After counting, resuspend the cells in the specific medium and adjust the cell density to 2.5×10^5 cells/mL.

After the gel has solidified, remove the 96-well plate and add 100 μL of cell suspension per well with MatrixGel-coated wells. Repeat this step for three wells. After marking, place the 96-well plate in a 37°C incubator for 2 hours of incubation.

Observe and photograph the formation of blood vessels under a 100x microscope.

In the optimal wells for blood vessel formation, carefully aspirate the medium and stain according to the Calcein AM kit instructions. Then, photograph and record using a fluorescence microscope.

3.1.3 Experimental Results

Figure 1 shows that PUMC-HUVEC-T1 cells can form obvious lumen-like structures on Matrigel (G4130/G4131). The actual effects may vary depending on experimental conditions such as cell type, status, and seeding density. Experimental conditions can be adjusted based on preliminary experimental results to achieve the best tube-forming effect.

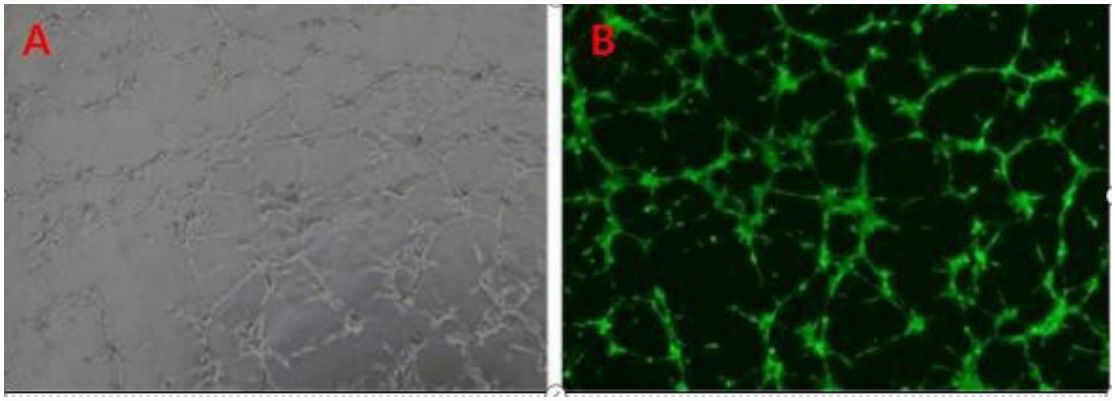


Figure 1. Brightfield (A) and fluorescence (B) images (100X) of PUMC-HUVEC-T1 vascular formation using Matrigel.

3.2 Tumor Cell TranII Invasion Assay (Using HT-1080 Cells as an Example)

3.2.1 Experimental Materials

MatrixGel MX0314/MX1314)

HT-1080 cells (STCC12701P/G)

HT-1080 cell-specific medium

TranII plates

PBS

DMEM medium

Recombinant Trypsin-EDTA Solution

without Phenol Red Universal tissue fixative

Crystal violet staining solution

200 µL pipette tips

1.6mL centrifuge tubes

1.7Pipette (SPIP-200)

3.2.2 Steps.

Place the MatrixGel in an ice box and store it in a 4°C refrigerator overnight to allow it to thaw slowly.Pre-cool the required 200 µL pipette tips and 1.5 mL centrifuge tubes in the 4°C refrigerator.

After thawing, vortex the vial or use pre-cooled pipettes/tips to gently mix the MatrixGel until uniform.Ensure that the MatrixGel remains on ice throughout the experiment.

Dilute the MatrixGel with PBS or serum-free DMEM medium at a 1:8 ratio in a 1.5 mL centrifuge tube (the dilution ratio can be adjusted based on experimental results to achieve an optimal cell penetration concentration).

Carefully pipette 80 µL of the diluted MatrixGel to evenly cover the surface of the upper chamber's polycarbonate membrane.Ensure the pipette tip is perpendicular to the membrane and add the MatrixGel slowly to avoid puncturing the membrane.The amount of MatrixGel added should be sufficient to coat the membrane without excess.

Place the chamber in the plate and incubate in a 37°C incubator for at least 2 hours.Remove any unbound MatrixGel and add 100 µL of serum-free medium to the chamber.

Incubate in a 37°C incubator for 30 minutes for hydration.

After hydration, check if any liquid has passed through the chamber into the lower chamber. If not, proceed with cell seeding. Digest the pre-cultured HT-1080 cells using recombinant trypsin, centrifuge, resuspend, and count the cells.Dilute the cells to concentrations of 5×10^5 cells/mL and 1.5×10^6 cells/mL using serum-free DMEM (adjust concentrations as needed for the experiment).

Add 500 µL of complete medium containing 20% FBS to the lower chamber of the Tranll plate. Carefully place the chamber into the well using forceps.

Add 200 µL of the cell suspension to the Tranll chamber.

Incubate the Tranll plate in a 37°C, 5% CO2 incubator for 24-48 hours.

Note:Avoid bubble formation between the lower medium and the chamber, as bubbles can affect the chemotactic effect. If large bubbles form, lift the chamber to remove them and then reinsert it into the plate.

Ensure even cell seeding by slowly adding the cells along the chamber wall.

Consider the impact of experimental treatments on cell proliferation when selecting time points. After incubation, remove the chamber and discard the medium.

Gently wipe off the MatrixGel and cells inside the upper chamber using a cotton swab.

Add 600 µL of universal tissue fixative to a new 24-well plate and place the chamber in the fixative for 20-30 minutes. Remove the fixative and stain the cells with crystal violet staining solution for 5-10 minutes.Discard the staining solution and wash the surface with PBS three times to remove excess stain.Use a cotton swab to wipe off cells on the seeding side of the chamber.

3.2.3 Experimental Results

Figure 2 shows that after culturing for 24 hours, HT-1080 cells exhibited significant invasion in MatrixGel (MX0314). Moreover, as the number of seeded cells increased, the number of invading cells also significantly increased.

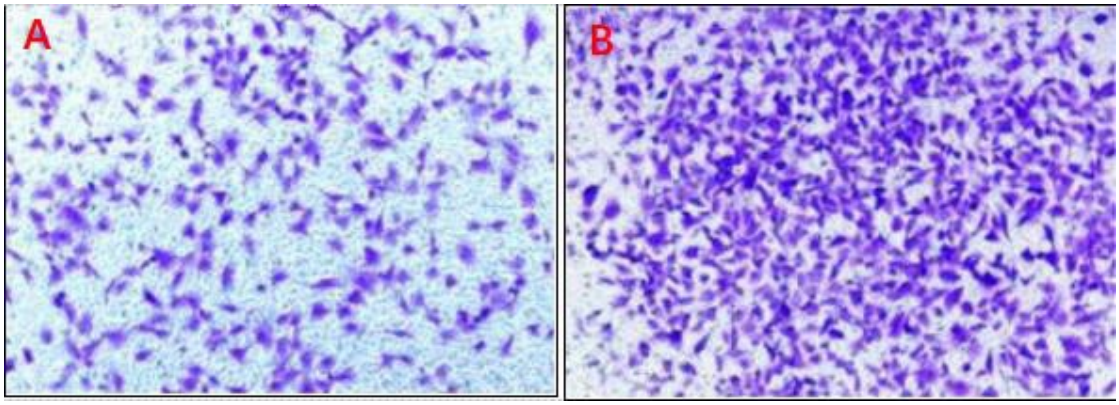


Figure 2. Images of Tranll invasion assay using MatrixGel (100X).
HT-1080 cells were seeded at densities of 1×10^5 cells/well (A) and 3×10^5 cells/well (B).

3.3 Tumor Formation Experiment (Using Daudi and Ishikawa Cells as Examples)

3.3.1 Experimental Materials

MatrixGel (MX0314/MX1314)

Daudi cells (STCC10903P/G)

Ishikawa cells

Daudi cell-specific medium (STCC10903P/G)

Recombinant Trypsin-EDTA Solution without phenol Red

200 µL pipette tips

1.5 mL centrifuge tubes

Pipette (SPIP-200)

3.3.2 Experimental Procedure

After purchasing, house mice at 5 mice per cage in an SPF-grade animal facility using IVC cages.Maintain the

room temperature at 22±2°C and relative humidity at 40%-60%.Provide free access to water and food.
Allow 3 days of acclimatization before starting the experiment.
For subcutaneous tumor formation experiments, typically use 4-8-week-old mice.Ethical guidelines usually require that the number of tumor cells inoculated should not exceed 1×10^7 cells/mouse.

Place the MatrixGel in an ice box and store it in a 4°C refrigerator overnight to allow it to thaw slowly.Pre-cool the required 200 µL pipette tips and 1.5 mL centrifuge tubes in the 4°C refrigerator.

After thawing, vortex the vial or use pre-cooled pipettes/tips to gently mix the MatrixGel until uniform.Ensure that the MatrixGel remains on ice throughout the experiment.

Thaw and culture the cells to be inoculated in advance.When the cells are in optimal condition, digest them using recombinant trypsin.
Stop the digestion using the specific medium, mix well, and centrifuge at 1000 rpm for 5 minutes.Discard the supernatant and resuspend the cells in serum-free medium, adjusting the cell concentration as required for the experiment.

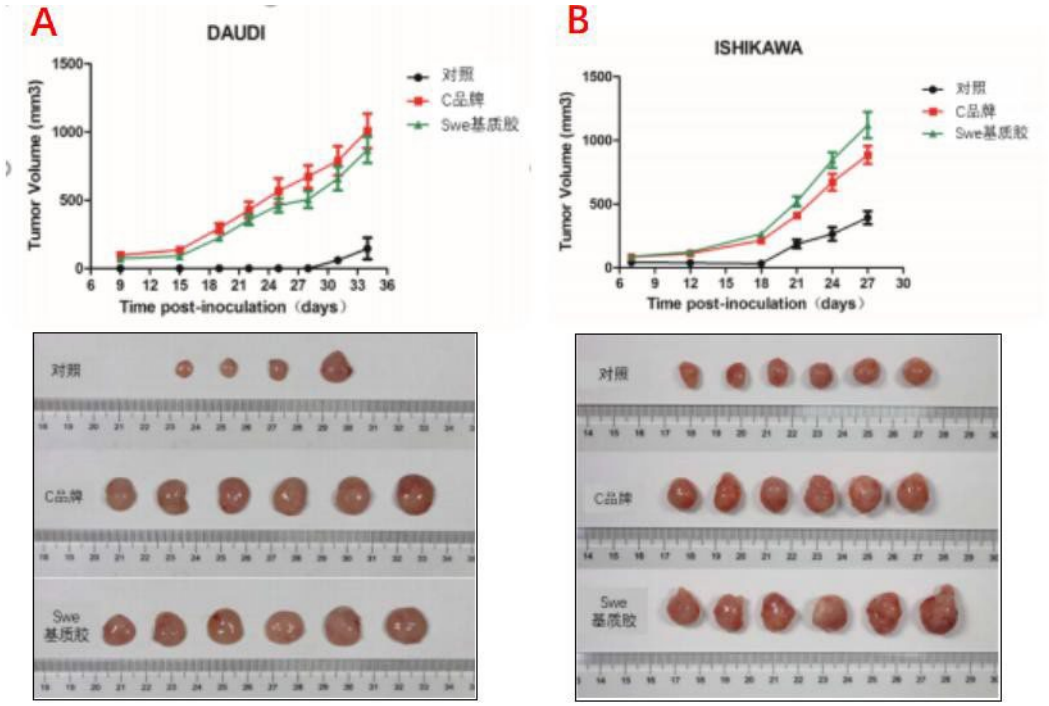
Mix the cell suspension with MatrixGel at a 1:1 ratio.The inoculation volume should be controlled at 100-200 µL/mouse.

Restrain the mouse and shave the hair on the right flank (not required for nude mice).Disinfect the shaved area with an alcohol swab.Use a pre-cooled insulin syringe to aspirate the mixture of MatrixGel and cells.Subcutaneously inject 100-200 µL of the mixture into the right flank.Slowly withdraw the needle and ensure no leakage before returning the mouse to its cage.

Regularly observe the mice for tumor growth and monitor tumor volume.
Tumors typically appear 7-14 days after inoculation, but some may require observation for up to 30 days.ZTake photos and collect samples as needed based on experimental requirements.

3.3.3 Experimental Results

Figure 3 shows that MatrixGel (MX0314/MX1314) significantly promotes tumor formation in difficult-to-form tumor cells, such as Daudi and Ishikawa cells.



3.4 Organoid Culture Experiment (Using Mouse Small Intestine Organoids as an Example)

3.4.1 Experimental Materials

MatrixGel (MX2314/MX3314)

C57BL/6 mice

DPBS (G4200-500ML)

Bovine serum albumin (BSA) (GC305006-100g)

Ethylenediaminetetraacetic acid (EDTA) (GC202001-500g)

Animal eye scissors (QX1030) Animal surgical blade #10 (QXJZ-10) 1000 µL pipette tips (TP-1000-C)

5 mL pipette tips (TP-5001L-F)

15 mL centrifuge tubes (EP-1500-BJ) Pipette (SPIP-1000)

Pipette (SPIP-5000) Cell strainer (G6016)

24-well plate (CCP-24H)

3.4.2 Experimental Procedure

Place the MatrixGel in an ice box and store it in a 4°C refrigerator overnight to allow it to thaw slowly.Pre-cool the

required pipette tips and centrifuge tubes in the 4°C refrigerator.After thawing, vortex the vial or use pre-cooled pipettes/tips to gently mix the MatrixGel until uniform.
Ensure that the MatrixGel remains on ice throughout the experiment.

Anesthetize the C57BL/6 mouse by intraperitoneal injection and euthanize by cervical dislocation.Soak the mouse in 75% ethanol for 10 minutes.Dissect the mouse and locate the pylorus of the stomach.Excise a 5 cm segment of the small intestine below the pylorus and another 5 cm segment above the cecum.

Immediately place the intestine in ice-cold DPBS and carefully remove residual mesentery and blood vessels.Use a 10 mL syringe to gently flush the intestinal lumen with ice-cold DPBS from the pyloric end to remove intestinal contents.
Repeat the flushing at least 3 times to ensure the lumen is clean.

Use eye scissors to carefully cut the intestine longitudinally and gently scrape off the villi using a surgical blade.Cut the scraped intestine into 2 mm segments and collect them in a 15 mL centrifuge tube.Add 10 mL of ice-cold DPBS to the tube, gently pipette to mix, and then discard the DPBS.

Repeat the above steps until the supernatant becomes clear, then discard the supernatant.

Add 10 mL of 5 mmol/L EDTA solution to the 15 mL centrifuge tube and gently pipette to mix.Incubate the tube in a 4°C refrigerator for 30 minutes.

Gently pipette the suspension repeatedly.
Examine the suspension under a microscope to confirm the presence of crypt-like structures.Filter the suspension through a 70 μm cell strainer into a new 15 mL centrifuge tube.
Centrifuge at 4°C, 1200 rpm for 5 minutes, and discard the supernatant.Resuspend the pellet in 10 mL of 0.1% BSA/DPBS solution.

Repeat the centrifugation and resuspension steps 3 times.

After the final resuspension, take 10 μL of the suspension for counting to calculate the total number of crypts.Centrifuge at 4°C, 1200 rpm for 5 minutes, and discard as much supernatant as possible.

Calculate the required volume of MatrixGel based on 1 μL of MatrixGel per 12.5 crypts.Resuspend the crypts in MatrixGel and gently pipette to mix, avoiding bubbles.Perform this step on ice to prevent premature gelation.

Pipette 50 μL of the suspension into the center of each well of a pre-warmed 24-well plate.Incubate the plate at 37°C, 5% CO2 for 30 minutes to allow the MatrixGel to solidify.

After the MatrixGel solidifies, add 500 μL of organoid growth medium to each well.Incubate the plate at 37°C, 5% CO2 and observe the organoid growth every 2-3 days.Replace the medium regularly and take photos to document

the growth

After 7-10 days or when the organoid center turns dark, aspirate the medium from the wells.Add 1 mL of ice-cold DPBS to each well and incubate for 1 minute.Gently dislodge the MatrixGel and pipette to break it up.Use a 1 mL syringe with a 27½ G needle to aspirate the suspension.

Repeat the above steps and transfer the suspension to a 5 mL centrifuge tube.
Centrifuge at 4°C, 1200 rpm for 5 minutes, and discard the supernatant.
Resuspend the pellet and passage at a 1:3 ratio.Repeat the seeding and culture steps as described above.

3.4.3 Experimental Results

Figure 4 shows that when using MatrixGel (MX2314/MX3314) for culturing mouse small intestine organoids, the organoids exhibit strong viability. By day 7, distinct crypt structures are observed, and the organoids maintain normal morphology and proliferation after passaging.

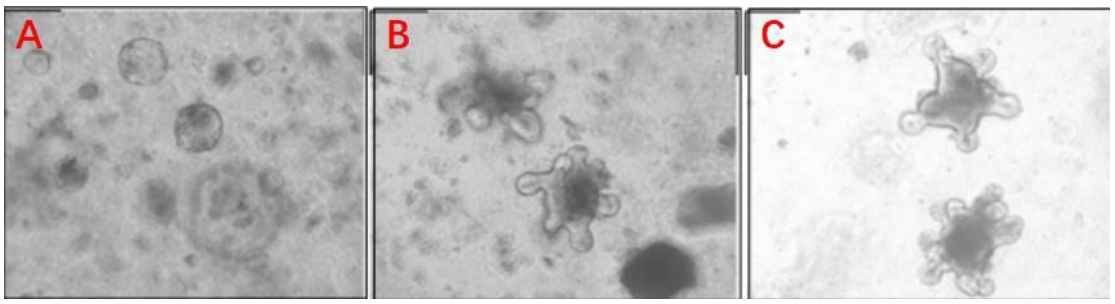


Figure 4. Images of C57BL/6 mouse small intestine organoid culture and passaging using MatrixGel (100X). (A) Day 3 of culture, (B) Day 7 of culture, (C) After passaging.

3.5 3D Spheroid Formation Experiment (Using HCT116 and NCI-H1975 Cells as Examples)

3.5.1 Experimental Materials

MatrixGel (MX2314/MX3314)
HCT116 cells (STCC10803P/G) NCI-H1975 cells (STCC10204P/G)
96-well plate (CCP-96H)
200 µL pipette tips (TP-200-C)
HCT116 cell-specific medium (GZ10803-500ML)
NCI-H1975 cell-specific medium (GZ10204-500ML)
Recombinant Trypsin-EDTA Solution without Phenol Red (G4022-100ML)

3.5.2 Experimental Procedure

Preparation of MatrixGel:

Place the MatrixGel in an ice box and store it in a 4°C refrigerator overnight to allow it to thaw slowly.Pre-cool the required 200 µL pipette tips and 96-well plate in the 4°C refrigerator.

After thawing, vortex the vial or use pre-cooled pipettes/tips to gently mix the MatrixGel until uniform.Ensure that the MatrixGel remains on ice throughout the experiment.

Place the 96-well plate on ice and add 50 µL of MatrixGel to each well.

Ensure the pipette tip is perpendicular to the well to avoid bubble formation.

Gently shake the plate to evenly distribute the MatrixGel.Incubate the plate at 37°C for 45-60 minutes to allow the MatrixGel to solidify.

Digest the pre-cultured HCT116 and NCI-H1975 cells using recombinant trypsin.Centrifuge, resuspend, and count the cells.

Adjust the cell density using the cell-specific medium (adjust as needed for the experiment).

After the MatrixGel solidifies, seed 1000 cells per well into the 96-well plate.

Incubate the plate at 37°C, 5% CO_2.

Replace the medium every 2 days and observe spheroid formation.Take photos to document the process.Spheroids typically form within 5-7 days.

Figure 5 shows that MatrixGel (MX2314/MX3314) significantly promotes the formation of spheroids in HCT116 and NCI-H1975 cells

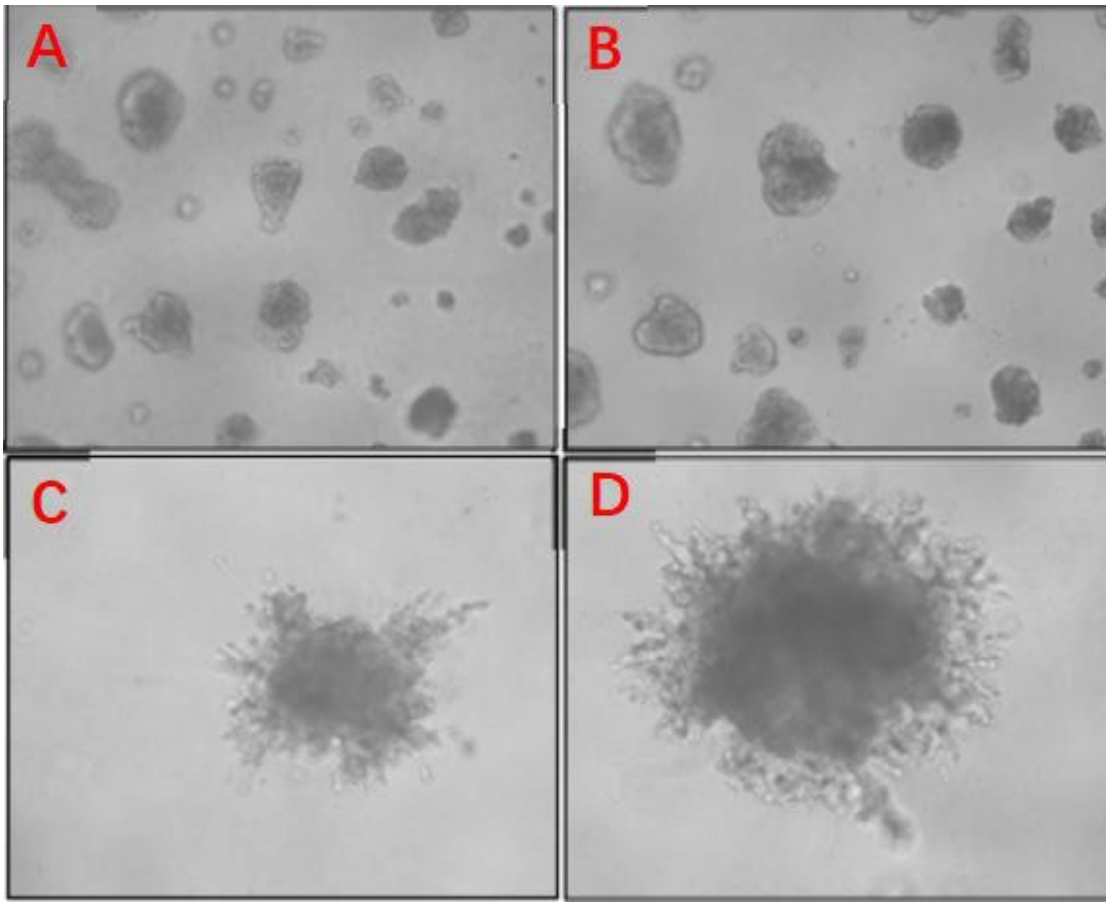


Figure 5. Photographs of 3D spheroid formation experiments using MatrixGel with HCT116 cells and NCI-H1975 cells.

(A)(B) HCT116 cells, (C)(D) NCI-H1975 cells, (A)(C) Cultured for 4 days, (B)(D) Cultured for 7 days.

4 Common Questions About MatrixGel

4.1 Arrival and Storage of MatrixGel

4.1.1 Precautions Upon Arrival of MatrixGel

Upon arrival of the WeMatrixGel, it is necessary to confirm whether there is a sufficient amount of dry ice in the packaging box, check whether the gel surface is basically level, and inspect the sidewall and opening of the bottle for any signs of gel melting to ensure the quality of transportation. If any issues are found, please take photos for record and provide feedback promptly.

4.1.2 Storage of MatrixGel

After receiving the MatrixGel product and confirming that it arrived in good condition, store it in a -20°C refrigerator. For long-term storage, it can be placed in a -80°C refrigerator. Do not store MatrixGel in a self-defrosting refrigerator to avoidaffecting product performance due to freeze-thaw cycles. After the first thaw, aliquot and store the product to avoid repeated freeze-thaw cycles.

4.2 Thawing and Aliquoting of MatrixGel

4.2.1 How to Thaw MatrixGel and Thawing Time

Thawing Method: Submerge the entire bottle of MatrixGel in a crushed ice box, then place the ice box in a 4°C refrigerator. It is recommended to place it near the back of the refrigerator and let it thaw overnight. Ensure there is sufficient crushed ice. After

thawing, rotate the bottle to check if the MatrixGel has completely melted, confirming there are no ice crystals or gel residues.

Thawing Time: It is recommended to thaw overnight. High protein concentration MatrixGel may require more time. For pre-aliquoted small amounts of MatrixGel, thaw at 4°C for 1 hour (for 1 mL) before use.

After Thawing: MatrixGel with phenol red should appear as a red liquid after thawing, while those without phenol red should appear as white or light yellow liquid. The higher the protein concentration, the more viscous the liquid will be after thawing.

4.2.2 MatrixGel May Still Solidify in the Refrigerator

Due to inaccurate refrigerator temperatures, especially in older refrigerators, the temperature can rise rapidly when the door is opened, sometimes exceeding 10°C. Therefore, in most cases, solidification is caused by excessively high refrigerator temperatures. It is recommended to place the MatrixGel on crushed ice and then place it in a 4°C refrigerator for thawing.

Additionally, the gelation of MatrixGel is not only temperature-dependent but also influenced by its protein concentration. Higher concentrations are more temperature-sensitive and more prone to solidification.

If your MatrixGel has solidified, first confirm the product's catalog number and concentration. Next, check the temperature of the refrigerator used for thawing and the duration of storage in the refrigerator. If solidification occurs, the following solutions can be applied:

For standard concentration products (8-12 mg/mL): If the refrigerator temperature is 6-10°C or the product is placed near the door for about a day, transfer the product to a 0±1°C environment for depolymerization for approximately 4 hours. It is recommended to check every 2 hours, and the product should return to a liquid state.

For high-concentration products (18-22 mg/mL): If the refrigerator temperature is 4-8°C or the product is placed near the door for about a day, transfer the product to a 0±1°C environment for depolymerization. It is recommended to check every 2 hours. The product should return to a liquid state, but this may take longer. The exact time cannot be provided due to

batch-to-batch concentration variations.

After the product returns to a liquid state, use it immediately or aliquot and store it frozen. For future use, thaw it on crushed ice to avoid similar issues.

4.2.3 Why Does MatrixGel Form Gels During Thawing?

MatrixGel is highly temperature-sensitive and begins to gel at temperatures above 10°C. Gelling occurs even faster at temperatures above 22°C. If the product arrives in normal condition, this phenomenon is likely due to improper thawing procedures or an increase in ambient temperature during thawing, causing partial gelation. Always follow the thawing protocol strictly and ensure a low-temperature environment during thawing.

4.2.4 Can Gelled MatrixGel Be Thawed and Reused?

You can attempt to thaw gelled MatrixGel by burying it in crushed ice and placing it in a 4°C refrigerator for 24 to 48 hours, then observe if it melts. Note that the performance of the MatrixGel after this treatment needs to be validated.

4.2.5 How to Aliquot MatrixGel

Repeated freeze-thaw cycles can affect the performance of MatrixGel. Therefore, it is recommended to aliquot the gel based on your single-use volume. Please note that all consumables in contact with MatrixGel, such as pipette tips and tubes, must be pre-chilled, and the procedure should be performed aseptically on ice. Avoid touching the bottom of the bottle when aspirating, and avoid dispensing too quickly or with excessive force.

If using a pipette, for example, when dispensing 5 mL, aspirate 6 mL and stop dispensing when 1 mL remains in the pipette. If using an automatic pipettor, aspirate by pressing to the second stop and dispense by pressing to the first stop.

Aliquoted MatrixGel can be stored in a -20°C or -80°C freezer. It is recommended to retain the bottle label or record the catalog number and batch number for future reference and tracking.

4.3 Characteristics and Use of MatrixGel

4.3.1 Characteristics of MatrixGel

MatrixGel gels at 37°C and remains in a liquid state at 4°C. It is extracted from the EHS tumor of mice and primarily includes laminin, type IV collagen, nidogen, perlecan, and some growth factors. At temperatures between 22°C and 37°C, covalent bonds between macromolecules can combine, prompting MatrixGel to form a gel. At lower temperatures (e.g., 4°C), there is not enough energy to promote covalent bond formation, so MatrixGel remains in a liquid state. The higher the protein concentration of MatrixGel, the more viscous it is. In addition to viscosity due to high concentration, the state of MatrixGel is also related to temperature changes and storage conditions during transportation. Dry ice should be used for refrigeration throughout transportation. If the refrigerator where MatrixGel is stored has an automatic defrost function, the temperature may rise during defrosting, potentially causing MatrixGel to gel. Therefore, avoid storing MatrixGel in such refrigerators.

4.3.2 Variations in MatrixGel Color

matrix gel may undergo color changes during the freeze-thaw process. This is due to the interaction of the bicarbonate buffer, carbon dioxide, and phenol red in the product. The color may shift from light yellow to deep red. This color change will disappear under 5% CO2 equilibration and is a normal phenomenon that does not affect the product's functionality. For the phenol red-free version, which appears white or light yellow, this phenomenon does not occur.

4.3.3 Dilution of MatrixGel

Generally, it can be diluted with pre-chilled serum-free medium or PBS at pH 7.4. Due to concentration variations between batches, it is recommended to dilute to a specific working final concentration rather than using a ratio or dilution factor.

Experimental Types	Tube Formation Assay	Invasion Assay	Organoid Culture	3D Culture	In Vivo Tumorigenesis Assay
MatrixGelDilution Ratio	Stock Solution （can be diluted 1:1 for use）	1:8	Stock Solution	Stock Solution	1:1
Recommended MatrixGel Usage	96-well plate 50 μL/well	24-well plate 10 μL/well	24-well plate 50 μL/well	24-well plate 50 μL/well	50-100 μL/pc
Amount	24-well plate 200 μL/well		6-well plate 300 μL/well		

4.3.4 Amount of MatrixGel Needed for One Experiment and How to Dilute It

The amount of MatrixGel required depends on the experiment and the number of wells. We provide recommended MatrixGel usage amounts for the following experiments. You can calculate based on our data or prepare aliquots in advance

4.3.5 Handling of Flocculent Precipitate in Undiluted MatrixGel

If MatrixGel contains multiple protein components, protein precipitation may occur during freeze-thaw cycles. In such cases, after thawing MatrixGel, centrifuge it at low speed at 4°C to remove precipitates.

4.3.6 Working Concentration of MatrixGel

Gel Concentration: In experiments requiring MatrixGel gelation, the working concentration should be greater than 3 mg/mL.

Coating Concentration:

4.3.7 How to plate MatrixGel to maintain a flat liquid surface

When plating the gel, it should be added as vertically as possible. If obvious bubbles appear when the gel is just added, they can be gently poked with a pipette tip. Gently shake the plate from side to side to flatten the gel before placing it in an incubator to solidify the MatrixGel.

4.3.8 How to make the blood vessel network formed by cells look good in the tube formation assay

The cells can be starved using serum-free medium the day before the experiment, which will enhance the tube formation effect on the second day. Additionally, attention should be paid to the cell density, as too many or too few cells will affect the tube formation. (When using MatrixGel in a 96-well plate for the tube formation assay, the recommended cell inoculum for PUMC-HUVEC-T1 cells is 2.5x10^4 cells per well.)

4.3.9 Why doesn't MatrixGel solidify during the invasion assay

When the concentration of MatrixGel protein is less than 3 mg/mL, the gelation phenomenon cannot be clearly observed. Generally, after incubating at 37°C for 2 hours, the MatrixGel will form a layer of matrix protein film at the bottom of the chamber to simulate the extracellular matrix.

4.3.10 How to harvest cells from MatrixGel

Neutral protease is recommended for harvesting cells cultured in MatrixGel. Compared to trypsin, collagenase, or other proteolytic enzymes, neutral protease can more gently and effectively obtain a single-cell suspension without damaging the cells or cell surface proteins. For cells that need to be further cultured or tested, using neutral protease will not cause damage. Additionally, neutral protease can also be used for tissue dissociation. Since MatrixGel contains trace amounts of RNA, when performing RNA analysis, a control group with MatrixGel (without cells) should be set up. Alternatively, cells can be harvested by lowering the temperature to 4°C-6°C to depolymerize the MatrixGel, but this method requires a longer time and may cause some damage to the cells.

4.3.11 When performing tumorigenesis experiments with the addition of MatrixGel, why do the tumors in mice disappear after growing

The shrinking and subsequent enlargement of the tumor may be caused by an inflammatory response. On the one

hand, after the tumor is fixed subcutaneously by MatrixGel in the early stage, due to the extremely small injection volume, the mouse will first absorb the MatrixGel, and then some tumor cells may be absorbed by the mouse itself. You can continue to observe for 2 weeks. On the other hand, you also need to consider factors such as the tumorigenicity of the cells themselves, cell viability, and the amount of cell injection. If it may be caused by these factors, you need to verify again.

4.3.12 When cells or organoids are cultured using MatrixGel and need to undergo sectioning or immunohistochemical and immunofluorescence testing, how should they be fixed, and how can depolymerization be avoided

They can be fixed using 4% paraformaldehyde. To avoid depolymerization after fixation, 1% glutaraldehyde can be added for fixation.

4.3.13 How long can MatrixGel-coated culture plates be stored

It is recommended to use MatrixGel-coated culture plates on the same day. If storage is necessary, they can be stored in a 37°C incubator for up to 1 week. During storage, the surface of the MatrixGel should be evenly covered with serum-free medium to keep it moist.

4.3.14 How should unused MatrixGel be stored

MatrixGel that has been mixed with cell culture medium or buffer but not used during the experiment is not recommended for retention and continued use.

4.3.15 What aspects should be noted when using MatrixGel

All reagents and consumables that come into contact with MatrixGel should be pre-cooled, and the entire operation process should be performed on ice to ensure a low-temperature environment. MatrixGel is very sensitive to temperature and will begin to gel once it exceeds 10°C.