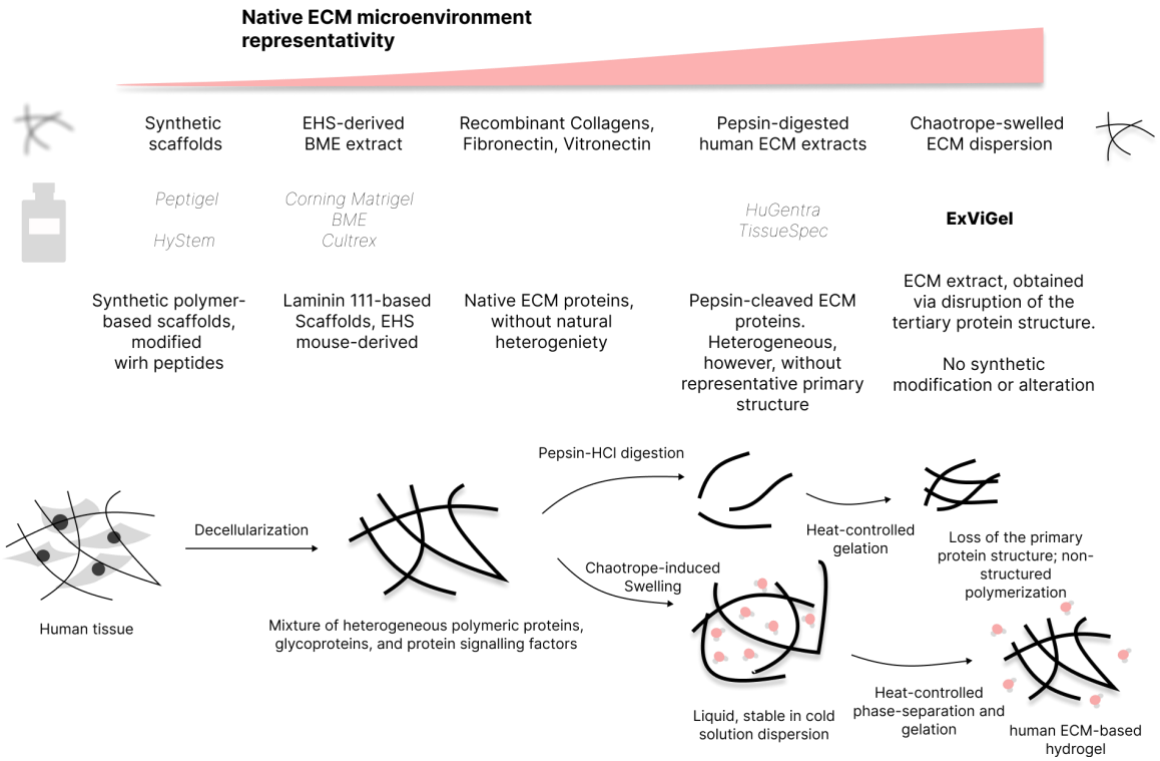
1st Edition by Preci LLC

What is a ExViGel?

ExViGel matrix is the first-in-class product (patent pending), non-enzymatically reconstituted extracellular matrix (ECM), derived from human sarcoma. Sarcoma is a mesenchymal tumor, frequently sizing few kilograms in the human body due to the uncontrolled accumulation of ECM. Previously described approaches to replicate the tissue ECM included synthetic matrices, pepsin-digested products or famously known Matrigel (EHS mouse-derived polymer protein mixture).

ExViGel production is based on the novel approach which improves typical chaotropic reconstitution of proteins, and allows insoluble proteins to form a stable colloid, which form the gel at +37°C. The sol state is stable at +4°C. Defined content of the ExViGel is described within Certificate of Analysis.



1st Edition by Preci LLC

Composition of the ExViGel:

- DMEM/F12, or L15 - as a basal medium
- Collagens I, III - >50%
- Laminin - >5%
- Fibronectin - >15%
- Elastin - >15%
- Different proteoglycans
- <10 ug of DNA/mg protein

Storage conditions:

- ExViGel Matrix should be stored in a freezer at -80°C.
- After thawing, ExViGel is stored at a temperature of 2 °C to 6 °C for no more than 3 days. Please, refrain from re-freezing the product.
- Constantly resuspend vigorously before use. Use the vortex, micropipette or ultrasonic batch (up to 2 minutes exposure) to keep the sol fully dispersed before use. There is a low chance of introducing air bubbles, but let the mixture stand for a few minutes if you notice too many of them.

General handling advises

1. Human ECM, ExViGel is a batch product of native human ECM proteins, including collagens. Due to the high complexity of the tertiary and quaternary structure of those, gel is highly turbid. Polymerization in driven by the phase separation of hydrogel and excessive water in its structure. Thus, hydrogel may contract significantly during in vitro experiments.
2. Please, use fluorescent live cell imaging dyes, that we advise in this handbook to monitor the cells state. Some dyes are incompatible with human-derived proteins, pay careful attention to ensure the consistency of your experience.
3. Pay attention to the clumps of hydrogel that may form if the gel is thawed incorrectly. Homogenize occasionally during the long-term (>30 minutes) standing to avoid the excessive flocculation.
4. Do NOT exceed 10°C storage temperature before use, keep on ice whenever possible.
5. Do NOT use cold medium, or dye solutions when manipulating the gel during cultivation. That may destroy the gel structure partially.
6. Use any commercially available DNase briefly, before doing cell lysis for PCR or DNA sequencing. Some extracellular DNA may appear in the mixture.

7. For better stability of the gel, if possible, before any manipulations let it stand in the incubator for 8-24 hours. That will ensure the strongest cross-linking of the proteins.
8. Do not hesitate to write a note to us at hi@preci.care to ask for additional advises, report any problems or ask for guidance. We are always here to help you in achieving your research goals.
9. To ensure the consistency of your experiments you can book the whole batch with no immediate purchase obligation.
10. Gel has a granular morphology when polymerized, typical observed granule size is 0,2-1 mm. This structure may be misinterpreted as a bacterial contamination. Every batch of ExViGel is tested for the sterility. Still ExViGel is a human-derived product and needs to be used in the proper cell biology environment (BSL-1 or BSL-2 facility is recommended).

Comparison to other products

Properties	ExViGel	EHS BME extracts (e.g. Corning Matrigel, BME)	Synthetic gels
Polymerization conditions	Temperature-controlled. Polymerizes at +37°C in 2 hours	Temperature-controlled. Polymerizes at +37°C in 1 hour.	Temperature-controlled or chemically controlled
Visual observation	Opacite, non-transparent, when polymerized	Slightly, transparent, when polymerized	Frequently opacite, non-transparent, when polymerized
Degree of cell-matrix interaction	High, very adhesive	Low, moderately adhesive	Different from product-to-product, unable to replicate the comprehensive cell-matrix interaction
Availability of protein content data	Yes	No	No
Availability of human ECM proteins	Yes	No	No
2D surface coating methodology	Usually done by centrifugation of the diluted gel on plasticware, or by phase-separation	By phase separation	Various
Preferred 3D culture methodology	Thick gel	Dome-like	Thick gel
In vitro/in vivo angiogenesis support	High, perfect for vascularization experiments	Moderate	None
Ability to manipulate the stiffness within cell biology lab infrastructure	Yes, explained further	No	No

Thawing time	Rapid, using warm water batch	Slow	Various
Customization availability	Per-request organ-specific ECM extraction. Addition of growth factors or structural proteins (e.g. collagen IV).	None	Rare
Cell Recovery Procedure	By cold PBS, collagenase or TrypLE	By cold PBS, collagenase or TrypLE	Product-dependent
Use within liquid handling equipment	Compatible with RT tips, not highly viscous	Only specialized liquid handling devices	Compatible with RT tips, frequently highly viscous

Common procedures

2D plasticware coating/overlay with ExViGel

Application

2D monolayer cell culture of immortalized and primary cell cultures. Cells seeded on top of the coated plasticware, when ExViGel formed the gel on surface.

Note

Homogenize the hydrogel well, when diluting it. Use ONLY cool PBS or serum-free medium to dilute. Make sure of an even distribution of sol within the coating solution. Diluted solution should stay cold when not used. Longer exposure to the surface may guarantee the better stability of the monolayer. Due to some cellular proteases and cell-matrix interactions the gel may degrade or form holes in the structure. In such cases consider lower dilution of the hydrogel.

Recommended Procedure:

1) Thaw the ExViGel vial rapidly using water batch, avoid secondary ice formation. Thawing should not take longer then 5 minutes, before the hydrogel is homogenized
2) Mix ExViGel intensively by pipetting 20-30 times, avoiding foaming. For better results use ultrasonic batch or vortex.
3) Calculate the required volume of the working solution based on the number of wells used and the data given in the table:

1st Edition by Preci LLC

Recommended volume and dilution of ExViGel for the homogeneous coating

Plasticware type	Area (cm^2)	Volume required for the layer, µL		
		20x	40x	80x
6-well plate	9.6	3000	4500	6000
12-well plate	3.5	1100	1600	2200
24-well plate	1.9	600	900	1150
48-well plate	1.1	300	450	400
96-well plate	0.32	100	150	200
Petri dish 35	8.8	2700	4000	5500
Petri dish 60	21.5	6600	9700	13500

1) Dilute ExViGel in the specified ration in chilled PBS or serum-free medium. E.g. 20x dilution means 25 µL of ExViGel for every 975 mL of dilutant. Mix carefully, use vortex or cooled ultrasonic bath for better results
2) Pour the required amount of the prepared solution into the wells
3) Shake the plate so that the liquid covers the entire surface of the well.
4) Centrifugate the plate at 4°C, 2500 g, 10 minutes.

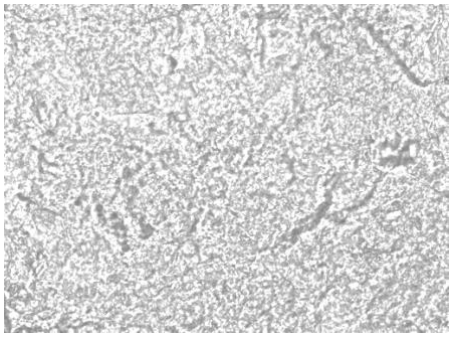
4a) Alternatively, use 10x or 20x dilution and incubate the well plate for at least overnight to obtain the homogeneous coating.

5) Incubate the plate at 37°C for at least 2 hours, do not agitate not to disturb the gel. If the dilution preferred is higher than 1/40, leave the plate overnight.
6) While the incubation continues, prepare the medium and the cells for further seeding.
7) After incubation, aspirate excess fluid from the wells.
8) Use ONLY warm (+37°C) cell suspension.
9) Start seeding the cells immediately after aspiration, by slowly pouring the suspension on the top of the formed layer. Do not allow the ExViGel to dry.

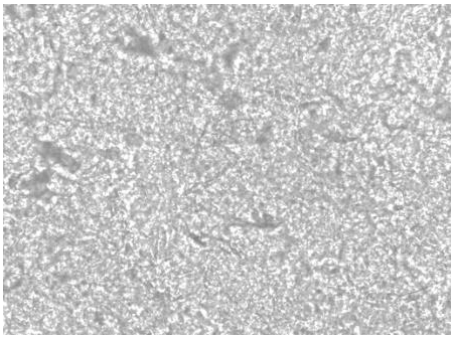
1st Edition by Preci LLC

Expected Result

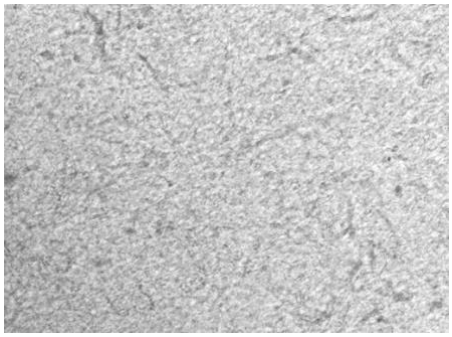
Microphotograph (10x of the formed coating)



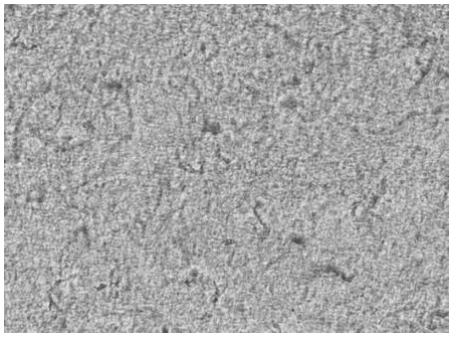
20x, thin layer



20x, thick layer



40x, thin layer



40x, thick layer

Hydrogel in dilution 10x

1st Edition by Preci LLC

Hydrogel in dilution 60x

Troubleshooting

Problem	Reason	Modification of the procedure
My hydrogel formed clumps in the vial of after the dilution	Improper homogenization	Homogenize the mixture vigorously using the pipette, vortex or ultrasonic equipment. Use only while cold, do not let the temperature increase during the homogenization
Gel sedimented unevenly on the well plate	Protein poorly suspending	Su spend the medium better
After cell addition, my coating was destroyed	The cell suspension was cold or/applied to the gel very quickly	Use warm cell suspension or/and applied to the gel slowly
After a few days of incubation my gel formed holes, became uneven, or floating	Poorly suspended protein or/and part of water evaporated	Suspend protein longer or/and slightly reduce incubation time
I do not need the whole vial of ExViGel for the coating of the well-plate	-	You can store gel at 4 degrees for several days
I cannot visualize the cells properly after using ExViGel coating	The dyes that were added are not recommended or the concentration of the dye was too high/too low	Use dyes which are recomended or/and raise your concentration/reduce your concentration

1st Edition by Preci LLC

2D monolayer overlay with ExViGel (sandwich-culture)

Application

Long-term 2D primary cell cultures.

Note

Homogenize the hydrogel well, when diluting it. Use ONLY cool PBS or serum-free medium to dilute. Make sure of an even distribution of sol within the coating solution. Diluted solution should stay cold when not used. Longer exposure to the surface may guarantee the better stability of the monolayer. Due to some cellular proteases and cell-matrix interactions the gel may degrade or form holes in the structure. In such cases consider lower dilution of the hydrogel.

Allow the gel to evenly coat the monolayer, do not disturb the well-plate for at least 24 hours ot ensure the homogeneity. Ensure that gel covers the whole surface of the well.

Due to the highly dense structure of the hydrogel, visualization of the cells may be complicated. Try lower dilution, if you desire to observe your cells constantly. Either use common compatible live-cell dyes that we advise further (Appendix A).

Recommended Procedure:

Expected Result

Microphotograph

Well-plate observation

Thick-layer cultures, low stiffness

Application

3D cultures, organ-on-chip application, vascularization experiments, ECM remodelling experiments.

Note

2D cultures may poorly represent the tissue complexity, including cell invasion, vascularization, angiogenesis. Classical Matrigel-based 3D cell cultures lack the proper invasion, stromal and vascular components. Spheroid cultures, which should lack attachment of cells to matrix cannot be obtained with ExViGel. Though, ExViGel can be used for cultivation of pre-formed spheroids to observe how cell-matrix interaction drive invasion. You can obtain think gels of various stiffness and density using ExViGel. Use the following methodology for low stiffness, low density studies. For cell imaging common compatible live-cell dyes that we advise further (Appendix A).

1st Edition by Preci LLC

Recommended Procedure:

1. Prepare the required number of cells in the appropriate medium.

2. Resuspend the cells in ExViGel. For low stiffness, dilutions of 1/3 to 1/9 are recommended. These dilutions are recommended to ensure that the gel has the required stiffness and amount of proteins and to allow visualization of your cell culture without using fluorescent dyes. Higher dilutions are recommended for easy observation in a microscope without dyes.

For example, 1 ml of cells in medium and 0.2 ml of gel.

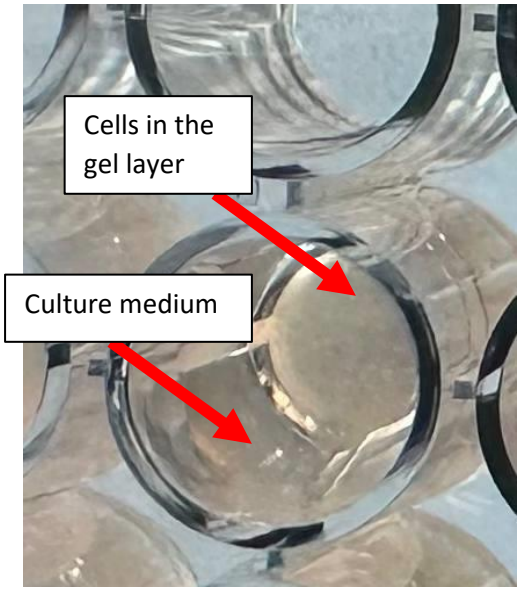
3. Add the required volume to the plate. For a 96-well plate, we recommend a volume of 200 µL.

4. Let your culture gel in the incubator for at least one day. Only then carefully replace/add warm culture medium along the walls of the well. Also, be careful when removing the medium without disturbing the unity of the layer.

5. After the required culture time, visualize the culture with the recommended dyes.

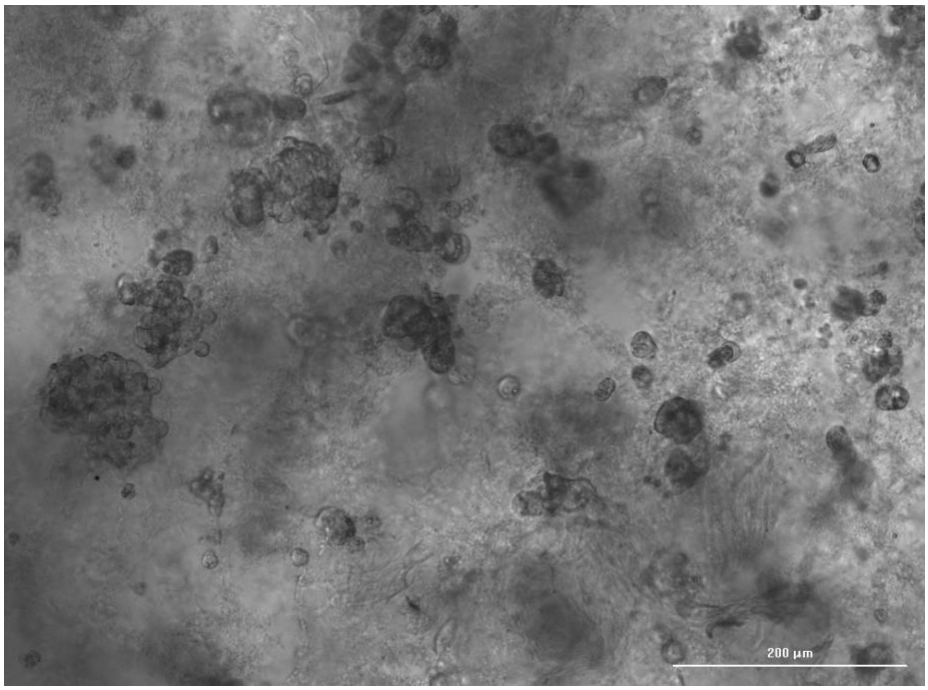
Expected Result

Well-plate observation



Microphotograph of cells in culture

1st Edition by Preci LLC



HepG2 in the ExViGel layer. Dilution 1/20

Thick-layer cultures, high stiffness

Application

3D cultures, organ-on-chip application, tissue engineering

Note

Use ONLY cold cell suspension, when mixing with ExViGel. Vortex or pipette vigorously before application of the suspension to the surface. Proper mixing is essential for the successful Thick-Gel cultures

Recommended Procedure

1. Prepare the required number of cells in the appropriate medium.

2. Resuspend the cells in ExViGel. For high stiffness, dilutions of 1/10 to 1/20 are recommended. For example, 1 mL of cells in medium and 0.1 mL of the gel.

3. Add the required volume to the plate. For a 96-well plate, we recommend a volume of 200 µL.

4. Let your culture gel in the incubator for at least one day. Only then carefully replace/add warm culture medium along the walls of the well. Also, be careful when removing the medium so as not to disturb the unity of the layer.

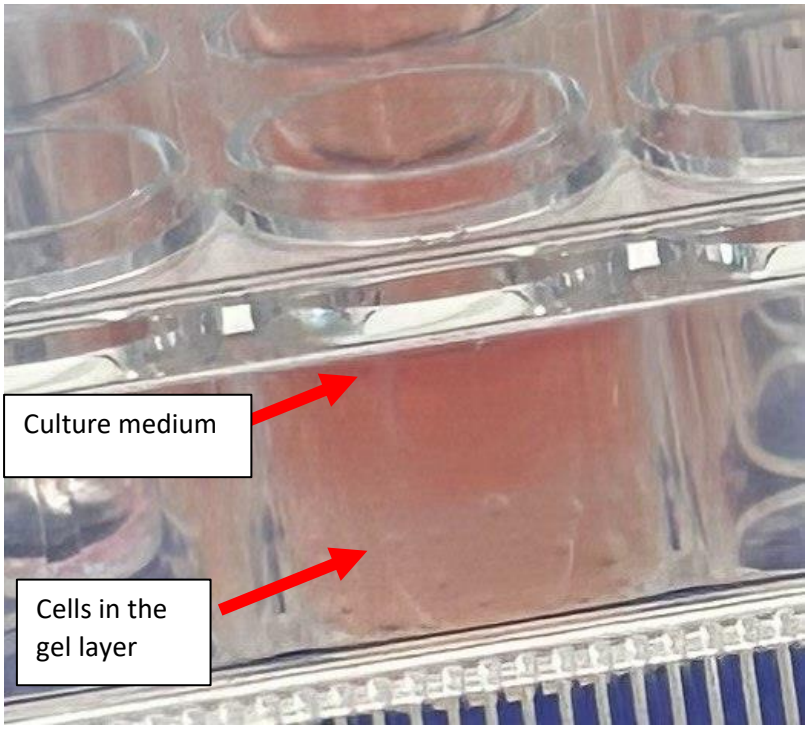
1st Edition by Preci LLC

5. After the required culture time, visualize the culture with the recommended dyes.

Expected Result

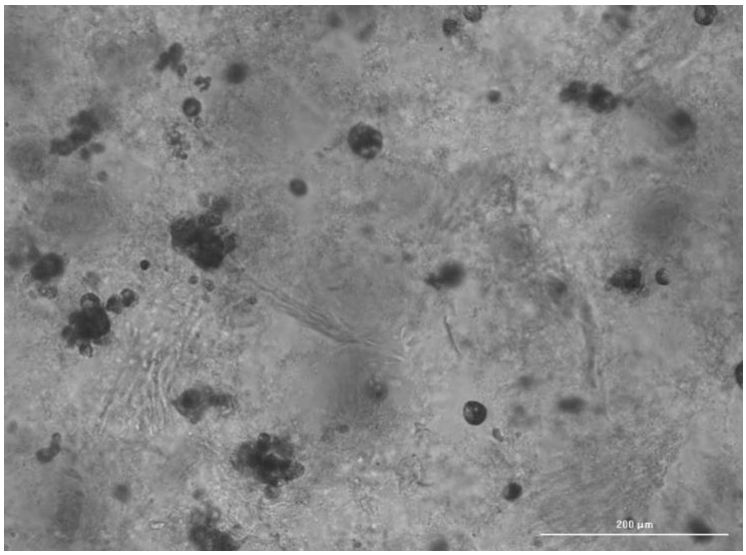
The 3D cell culture hydrogel system, utilizing ExViGel, simulates the microenvironment of human tissue. This network provides structural support and functional biochemical interactions to the surrounding cells. Additionally, it influences gene expression, causing varying levels of expression and enabling the expression of some genes that do not express in a 2D culture system.

Well-plate observation



Microphotograph of cells in culture

1st Edition by Preci LLC



HEP-G2 in the ExViGel layer. Dilution 1/7

Troubleshooting

Problem	Reason	Modification of the procedure
My hydrogel formed clumps in the vial of after the dilution	Improper homogenization	Homogenize the mixture vigorously using the pipette, vortex or ultrasonic equipment. Use only while cold, do not let the temperature increase during the homogenization
My hydrogel has disassembled	The culture medium was added/changed too quickly. Insufficient incubation time of cell culture in the gel	Add/change the medium slowly, along the wall of the well. The gel with cells should stand for at least a day without adding/replacing the medium when first applied
I see only precipitate instead of the dome	The gel was not vortexed and/or had been refrigerated (or kept warm) for a long time. The cells were not resuspended well enough with the gel. The cell culture with the gel was not kept in the incubator for enough time before changing the medium	Store and use the gel as recommended. Mix the cells well with the gel before adding to the well. The cells with the gel should be incubated for at least one day before adding/changing the medium.
After a few days of incubation my gel formed holes, became uneven, or floating	The culture medium was added/changed too quickly. Insufficient incubation time of cell culture in the gel	Change/add only warm medium along the well wall, slowly. Before adding/changing the medium, make sure that your cell gel has been incubated for at least a day until it is completely gelatinized

I cannot visualize the cells properly within ExViGel	The gel was diluted in the medium incorrectly (too dense gel). Your dye is not included in the list of recommended ones, or was used not according to the protocol	Make sure you used the dyes/gel according to the protocol (correct concentrations and dilutions). Check if your dyes are included in the list of recommended dyes

In vivo injection

Application

Tissue engineering, Xenograft cultures (immortalized or primary), in vivo disease modeling

Note

PRODUCT IS NOT INTENDED FOR MEDICAL OR DIAGNOSTIC USE. Only injection into non-human research objects is permitted.

Every ExViGel batch is qualified for the use in rodent models. No body weight loss, inflammation or any other noticeable side reactions in regular or nude tested animals are observed during subcutaneous injections in the volumes below 1 mL of ExViGel. Use with sterile consumables.

Use ONLY cold cell suspension, when mixing with ExViGel. Vortex or pipette vigorously before application of the suspension to the surface. Proper mixing is essential for the successful Thick-Gel cultures.

Use high concentrations and higher volumes of ExViGel for better implant longevity in PDX models.

Materials

1. Cells in high concentration
2. Cell culture medium (DMEM + 10 % FBS + 1% Antibiotic Antimycotic)
3. ExViGel
4. Reagents for anesthesia (2,2,2-Tribromoethanol 97%)
5. Sterile 1 ml insulin syringes with 27-gauge needles or sterile 1 ml syringes with 23-gauge needles

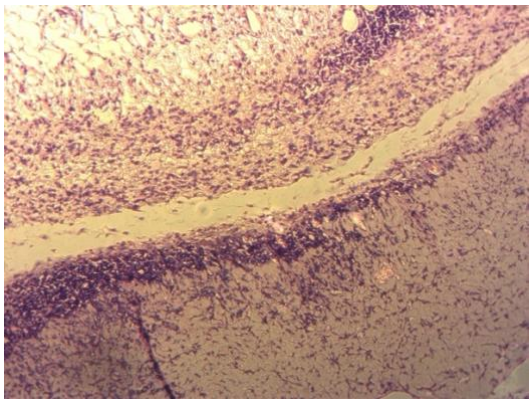
Recommended Procedure

Before the procedure: prepare all materials and cool to 4 °C.

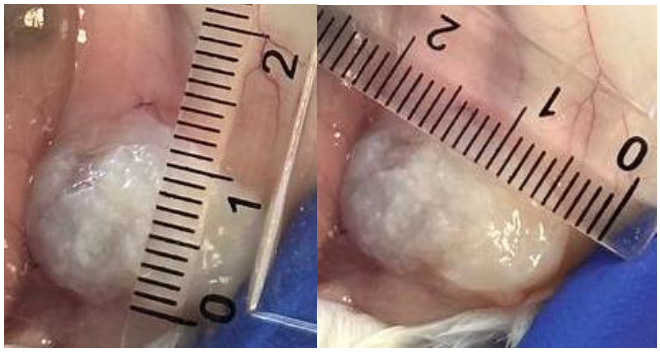
1. Thaw the gel in a water bath at approx. 30°C for 1-2 minutes. After thawing, vortex and vigorously resuspend the gel. Afterwards, store the gel in the refrigerator at 4°C
2. Prepare the cells that have been re-suspended in the cell medium. For tumor implantation applications, approximately $10*10^6$ cells/100 µL of cell suspension should be mixed with ExViGel, resulting in a final cell concentration of ~$5*10^6$ cells/100 µL.

3. The final cell suspensions of $5*10^6$ cells/100 µL in culture medium and ExViGel mix (1:1), should be kept on ice before injection.
4. The volume of the suspension to be injected varies depending on the purpose of the experiment. We recommend injecting the volume in between 0.1-0.5 mL for the simulation of tumor growth. An injection volume of 0.5 mL – 1 mL is recommended for in vivo angiogenesis studies and for patient-derived xenograft establishment.
5. After mixing ExViGel with a cell suspension, the mixture is injected into a mouse. The injection can be either orthotopic or subcutaneous, depending on the purpose of the experiment.

Implant photos, including histology



HE staining image of a mouse tumor. The photo shows angiogenesis and cell invasion into the ExViGel.



Size and general view of the tumor. Growth time 15 days, injected volume 0.7 ml

Expected Result

The expected results are the formation of new blood vessels (angiogenesis) and tumor growth in the mouse. For a better understanding, angiogenesis research requires models that better mimic the

multistep tumorigenesis of human cancer, from initial genetic disruption in individual cells to malignant progression in the proper tissue environment.

Appendix A

For proper imaging in the dense non-transparent hydrogels, it is recommended to use the common cell visualization dyes. We do not make preference to any supplier; any structurally equivalent dyes to listed should work well to the advised ones.

If you do not see your system in the listed dyes, consult with our specialist at hi@preci.care.

Application	Recommended	NOT Recommended
Nuclei Visualization (live-cell)	DAPI (4',6-diamidino-2-phenylindole), bisbenzimide	
Cytoskeleton Visualization	Any stains, except for taxol-based	Taxol (paclitaxel) and its analogoues
Cytoplasm Visualization (live cell)	Calcein AM	
Membrane Visualization	Steady Membrane Staining Kits	Concanavalin A
Dead cell staining (live-cell)	Propidium iodide	
Lipid droplets	Any Lipid Droplet Stains	

Appendix B

Application note. Culturing Cancer-Associated Fibroblasts (CAFs) using different culture systems of ExViGel

Cancer-Associated Fibroblasts display rapid behavior change within different ECM. The effects of ECMs can be segregated to ligand-specific (integrin-driven) and ligand-agnostic (stiffness-deriven). ExViGel is perfectly suited to drive both effects for more native physiology of CAFs. Higher cell differentiation from CAFs to myofibroblasts can be achieved using different cultivation systems.

Materials and Methods

- ExViGel
- Collagen 1 type
- BME
- CAFs
- Culture medium (DMEM + 10 % FBS + Antibiotic Antimycotic)
- Dye mix (PI, Calcein, DAPI)

1st Edition by Preci LLC

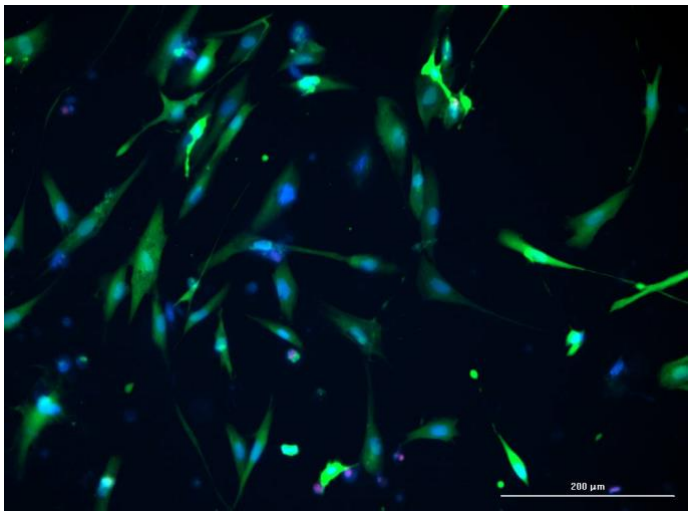
Procedure

1. The plate was coated with ExViGel according to the coating protocol above. The wells were coated with ExViGel of different batches, Corning and collagen. Different dilutions were used.

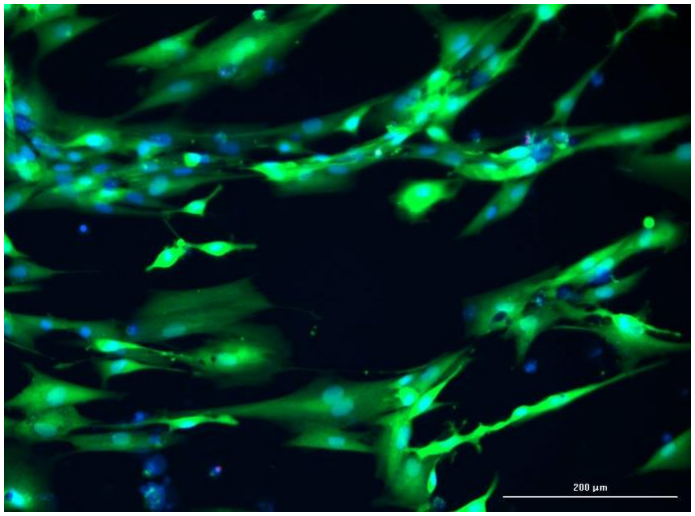
2. One day later, Cancer-Associated Fibroblasts (CAFs) were applied to the coating at a concentration of 5 thousand cells per well.

3. The cells were cultured for 3 days and then visualized using dyes (DAPI - nuclei, PI - dead cells, Calcein - live cells).

Results

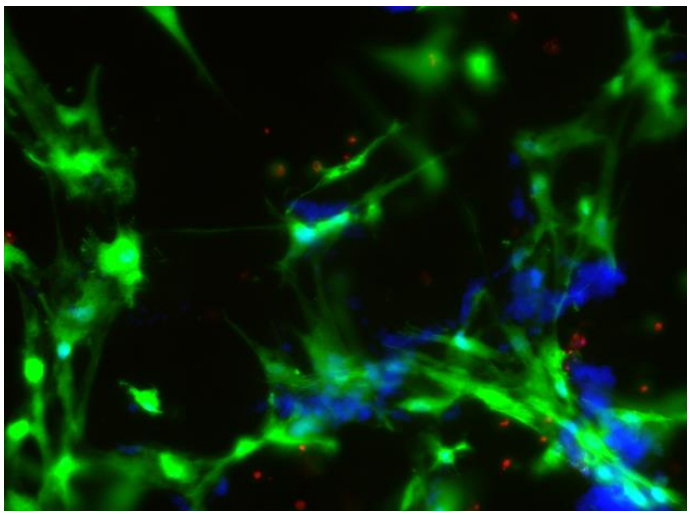


Cancer-associated fibroblasts (CAF) on a collagen coating. (Green - cytoplasm of living cells, blue - nuclei, red - dead cells)



1st Edition by Preci LLC

Cancer-associated fibroblasts (CAF) on the BME coating, dilution 1/30. (Green - cytoplasm of living cells, blue - nuclei, red - dead cells)



Cancer-associated fibroblasts (CAF) on the ExViGel coating at 1/30 dilution. (Green - cytoplasm of living cells, red - dead cells)

Appendix C

Application note. MDA-MB-231 xenograft culture using ExViGel

Materials and Methods

- SCID/beige female mouse
- MDA-MB-231 cells
- BME
- ExViGel
- Culture medium (RPMI with 10 % FBS, 100 U/mL of penicillin, and 100 µg/mL of streptomycin)
- 0.05 % trypsin-EDTA solution
- DMEM
- trypan blue

Procedure

5M of MDA-MB-231 cells were orthotopically inoculated into 4th right mammary fat pad. Cells had been cultured in RPMI (4.5 g/L glucose) with 10 % FBS, 100 U/mL of penicillin, and 100 µg/mL of streptomycin at 370C and 5 % CO2. Cells were harvested using 0.05 % trypsin-EDTA solution, centrifuged, and suspended in serum-free DMEM. Cells count and viability were accessed using a hemacytometer and trypan blue exclusion test. The final cells suspensions of 50×10^6/mL in DMEM and gel mixed (1:1), were kept on ice before injection.

SCID/beige female mice randomized into six groups:

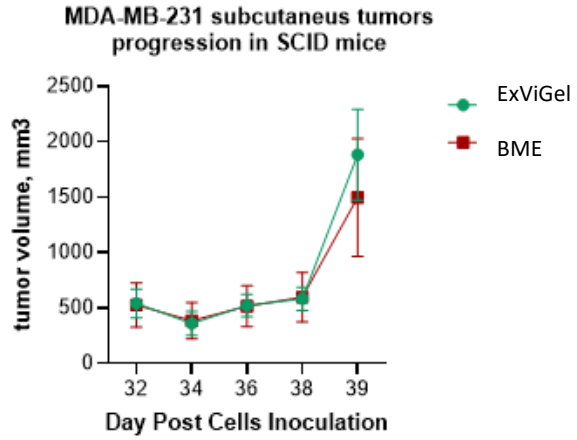
- MC MDA group (4T1 cells in BME).n=3

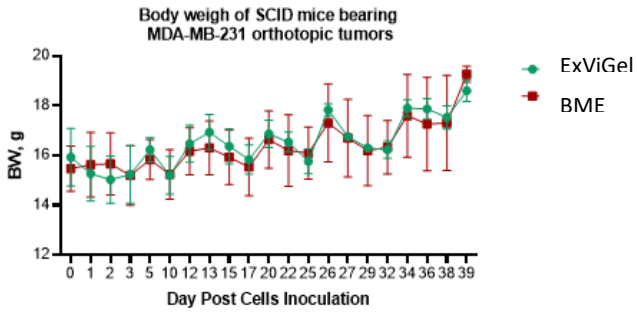
1st Edition by Preci LLC

- MP MDA group (4T1 cells in Matrix from PRECI). n=3
- Control MDA group (Matrix from PRECI without cells). n=3

Results

There was noticeable tumor growth in both cases: ExViGel and Matrigel. As a result, the tumor with ExViGel grew larger than the one with Matrigel at the same time. Importantly, no clinical signs of toxicity were observed in mice injected with the PRECI Matrix.





1st Edition by Preci LLC