Protocol for Matrigel Dome Culture with GelControl™ Inserts

A protocol for designing, seeding, culturing, imaging, transferring, and evaluating hydrogel dome constructs.

Recommended use
Use this protocol when evaluating GelControl™ inserts for workflows where hydrogel dome position, shape, handling, imaging access, and operator-to-operator consistency are important experimental variables. The protocol is especially relevant for Matrigel dome workflows, organoid or spheroid embedding, stromal co-culture models, interaction assays, and soft hydrogel constructs that are difficult to lift, transfer, or image consistently.

1. Workflow overview

GelControl™ inserts are designed to complement a soft gel ECM by providing a physical support structure for dome formation, culture, fixation, lifting, and transferring. The inserts contain an inner ring that defines the dome area, a thin microfiber grid beneath the hydrogel that enables lifting and transfer, wings that center the gel within the well, and a handle for forceps manipulation (Figure 1). See table 1 for physical specification.

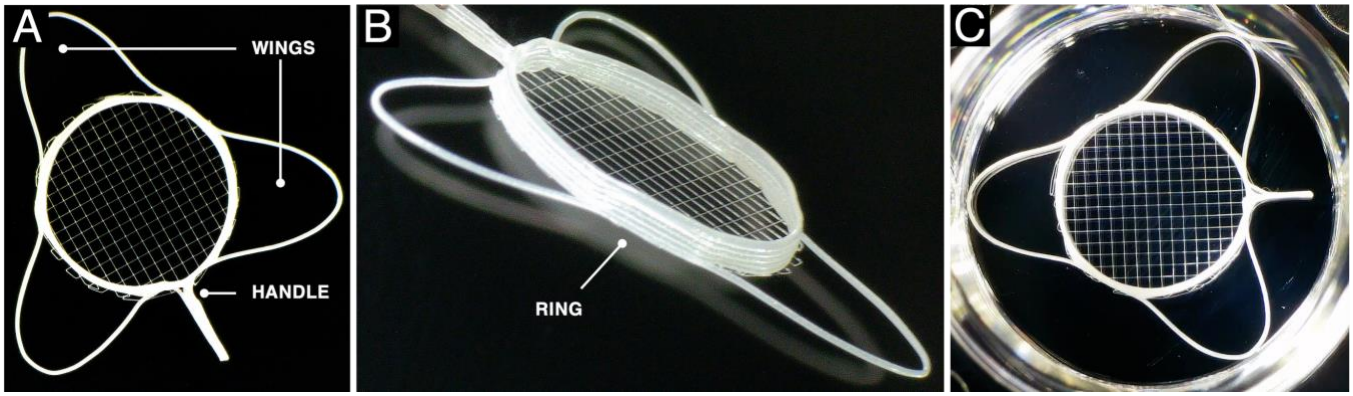

Figure 1: GelControl™ Insert design sized for 24-well plate. A) Top view of inserts highlighting the wing and handle. B) Isometric view of insert highlighting the inner ring portion that molds soft hydrogel shape. C) Top view of insert mounted inside a well plate. The wings press against the walls to keep hydrogel constructs centralized within the well.

Insert size	Outer diameter	Ring diameter	Insert Height	Grid fiber spacing	Grid fiber diameter
24-well	16.4 mm	8.0 mm	720 µm	500 µm	14 µm
48-well	10.8 mm	6.5 mm	720 µm	500 µm	14 µm

Table 1: Specifications of GelControl™ inserts.

Workflow step	User action	GelControl function	Recommended QC check
Plan experiment	Choose ECM concentration, cell density, dome volume, culture duration, and endpoints.	Ability to transfer intact cultures	Include matched controls during evaluation.
Prepare hydrogel-cell suspension	Keep Matrigel, tubes, and tips cold; lift cells, count them, and prepare cell-gel solution to target final concentrations.	GelControl inserts arrive sterilized and ready-to-use	Confirm cell suspension is homogeneous and bubble-free.
Seed dome into insert	Dispense the hydrogel-cell suspension into the inner ring.	Simplifies dome formation with consistent size and position	Check that the gel fills the ring evenly without bubbles.
Gelation and media addition	Allow gelation before gently adding warm complete medium pipetted against the wall.	Provides support during media addition and subsequent exchanges.	Confirm the dome remains attached and centered.
Culture and monitor	Perform media exchange and brightfield monitoring.	Domes supported and anchored during media changes. Monitor cultures via brightfield	Track dome position, and cellular phenotype via brightfield.
End culture	Fix, stain, and image intact domes, or perform other endpoint assays.	Transfer fragile constructs.	Compare endpoint results with matched controls

2. Materials and equipment

The table below lists the materials that were used to evaluate GelControl inserts in a workflow with Matrigel domes.

Category	Material	Purpose / notes
Gel support	GelControl™ inserts	Physical support, shape mold, and central positioning of Matrigel domes. The inserts also enable lifting and transfer of whole intact domes with minimal deformation
Cell culture plate	Sterile 24-well plate format compatible with GelControl inserts	Use the plate format supplied or specified by VivoTex. Matched control wells should use the same plate type where possible.
ECM	Corning® Matrigel® matrix	Prepare final target concentration after accounting for dilution by cells, medium, and additives. Keep cold before dispensing.
Cells or organoids	NIH 3T3 fibroblasts for example workflow; user-selected organoid or cell model for application work	Use healthy cultures and model-specific passage, viability, and dissociation requirements.
Basal medium and supplements	DMEM + 10% FBS + 1% penicillin-streptomycin for NIH 3T3 example	Replace with model-specific complete medium for organoids or primary cells.
Cold plasticware	Pre-chilled low-retention pipette tips, microcentrifuge tubes, conical tubes, reagent reservoirs, and tube racks	Minimize premature Matrigel gelation and reduce material loss during mixing.
Liquid handling	P200/P1000 pipettes, serological pipettes	Use slow, smooth pipetting to prevent bubbles and shear.
Handling tools	Sterile fine forceps; optional sterile spatula for unsupported controls	Forceps are used to grasp the GelControl handle during lifting or transfer.
Imaging	Inverted brightfield microscope; fluorescence microscope or high-content imager	Use consistent imaging settings and scale bars for supported and control domes.
Metabolic assay	alamarBlue™ Cell Viability Reagent or equivalent resazurin-based reagent	Measures relative metabolic activity; does not uniquely distinguish proliferation, cell number, apoptosis, necrosis, or metabolic state.
Endpoint staining	4% paraformaldehyde, PBS, 0.1% Triton X-100, Hoechst, phalloidin	Optional fixed-cell imaging workflow used in the application note.

3. Recommended starting parameters

Parameter	Starting values		Guidance
	24-well	48-well	
Dome volume	60-80 µL	35-45 µL	Select a volume that fills the inner ring without overflow.
Medium volume	1000 µL	400 µL	Ensure medium covers domes
Gelation time	20 minutes at 37°C		Confirm gelation for each ECM lot and experimental condition.
Matrigel concentrations	3.0 - 6.0 mg/mL		Optimize for model based on viability and endpoint assays
Cell densities	0.1-2.0 · 10^6 cells/mL		Use cell-type-specific density and viability requirements.

4. Safety, sterility, and biosafety requirements

This protocol is for research use only and is not intended for diagnostic, therapeutic, or clinical use. Users are responsible for following all institutional biosafety, chemical hygiene, waste-disposal, and training requirements applicable to their cell model, hydrogel, reagents, and assay endpoints.

GelControl Inserts
GelControl inserts are made of polycaprolactone, a semi-crystalline polyester polymer that is commonly used in the biomedical field for sutures, implants, and in tissue engineering applications (1). Use the inserts only as supplied and according to the intended research-use workflow. Do not resterilize, reuse, modify, or use damaged inserts unless those conditions have been independently validated. When handling GelControl inserts, use sterile technique to ensure successful use. Spray package with ethanol before placing inside a clean biosafety cabinet (BSC). Only use sterile tools for handling inserts. Use sterile or autoclaved forceps to minimize introduction of contaminants.

Matrigel
GelControl inserts are not supplied with Matrigel or other hydrogels. This protocol provides basic handling guidance for Matrigel as an example ECM hydrogel used with GelControl inserts. Please refer to the Matrigel specifications and protocols for more detailed information (2,3). Matrigel is a basement membrane matrix extracted from mouse sarcoma that is rich in extracellular proteins. Handle Matrigel using sterile technique and wearing appropriate PPE in a certified BSC. Maintain sterility of GelControl inserts, plates, pipette tips, tubes, media, and forceps throughout setup, seeding, culture maintenance, and transfer.

Mammalian cell culture, organoids, and other biological materials
Follow institutional biosafety requirements for the specific cell type, organoid model, source material, genetic modification status, infectious-agent status, and viral-vector use associated with the experiment. Users should determine the appropriate biosafety level and containment practices before beginning the protocol.

Handle all live cultures and culture waste as potentially biohazardous unless the institutional biosafety assessment specifies otherwise. Use appropriate PPE, aseptic technique, approved disinfectants, and validated decontamination procedures for the cell model and facility.

Chemical reagents
Fixatives, permeabilization reagents, stains, metabolic assay reagents, solvents, and endpoint assay materials may present chemical hazards. Review the relevant safety data sheets and follow institutional chemical hygiene procedures for handling, labeling, storage, exposure control, spill response, and disposal. Paraformaldehyde, Triton X-100, DMSO, fluorescent stains, and other assay reagents should be handled using appropriate PPE and facility-approved chemical controls. Prepare and dispose of chemical and biological waste according to institutional requirements and applicable regulations.

5. Before you start

Plan the comparisons with controls

- When starting to use GelControl inserts, compare GelControl-supported and conventional control domes from the same Matrigel-cell suspension, and keep their experimental conditions as similar as possible. Some common sources of variation include:
  - Evaporation and humidity differences in edge wells.
  - Well plates from different manufacturers may differ in geometry or surface treatment.
  - Pipetting technique – most seeding workflows are time-sensitive, and the order of pipetting affects the spread of results
- Interlace well positions across the plate to reduce position and timing effects.
- Plan primary endpoints before the experiment.

Prepare cold materials (day before)

- Thaw Matrigel in 2-8 ºC according to the supplier's recommendations.
- Perform calculations for Matrigel dilutions, record the required volumes and keep them available in the area by the biosafety cabinet (BSC). To see examples of the calculations refer to Appendix A. Example calculations for matrix concentration and cell density.

Prepare cells or organoids

- Use cultures with high viability.
- Cells can be either thawed from frozen, or passaged from expansion.

6. Procedure

Step 1. Prepare GelControl™ plates and control wells

1. Bring the GelControl plate or insert-containing well plate into the BSC while maintaining sterility.
2. Keep the plate closed until immediately before use.
3. Prepare matched control wells in the same plate type where possible. Label plate maps clearly to distinguish GelControl-supported domes from conventional control domes.
4. Assemble equipment and materials for Matrigel
   - Pre-chill tubes, pipette tips, and reservoirs that will contact Matrigel.
   - Keep Matrigel and Matrigel-containing mixtures cold until the moment of dome dispensing.
   - Note: Matrigel will start polymerization if any part of it reaches 10ºC.

Step 2. Prepare the cells for seeding

5. Harvest cells or organoids according to the model-specific protocol.
6. Count cells or organoid fragments and determine viability.
7. Prepare a concentrated suspension that will yield the desired final cell density after mixing with Matrigel.
8. Keep the suspension cold (if compatible with the cell model).

Step 3. Prepare the Matrigel-cell mixture

9. Prepare volumes recordings of stock Matrigel, cells, diluent, and additives required to reach the desired final matrix concentration and cell density.
10. Mix stock Matrigel with diluent gently by pipetting with pre-chilled tips and avoid bubbles.
11. Combine cells and Matrigel gently in a chilled tube. Mix until homogeneous without over-pipetting.
12. Note: Matrigel viscosity and gelation behavior change as the mixture warms. This may make large experiments with many domes more difficult to seed. Calibrate your containers and cooling equipment to best suit your workflow.

Step 4. Seed Matrigel domes

13. Using a pre-chilled pipette tip, aspirate the required dome volume. For the 24-well use 60 µL per dome; for 48-well use 40 µL per dome.
14. Seed the same Matrigel-cell suspension and the same dome volume for supported domes with GelControl inserts and conventional, unsupported, Matrigel domes.
15. For accurate evaluation of the effects of the inserts, interlace the seeding order and well position within the same plate to form matched controls.
16. Inspect each dome immediately for bubbles, overflow, incomplete filling, or off-center loading. Record any seeding defects.

GelControl Inserts

17. Position the pipette tip above the inner ring of the GelControl insert. Dispense solution slowly into the ring. The solution should fill the defined inner ring.
    - To improve pipetting control the pipette tip can be leaned against the top of the wall edge. Watch Demonstration
    - Avoid touching the microfiber grid with the pipette tip. Do not scrape or push against the grid. Watch Demonstration

Conventional Matrigel domes

18. Dispense Matrigel domes directly into matched wells.
19. Use consistent pipetting angle, dispense speed, and well location across controls.

Step 5. Gel the domes

20. Transfer the plate carefully to a humidified 37 °C incubator.
21. Allow domes to gel undisturbed for 20 minutes before media addition.
    - Some protocols advise turning Matrigel dome upside down for gelation to discourage cell attachment to the tissue culture plastic. GelControl inserts are compatible with this optional step, and reliably remain on the bottom of wells.
22. Do not add medium until domes are visibly gelled.

Step 6. Add complete culture medium

23. Warm complete medium to 37°C (or appropriate temperature for the cell model).
24. Gently add medium along the wall of each well, away from the dome. For the 24-well, add 1000 µL per well; for 48-well add 400 µL.
    - Handle plates with domes carefully between an incubator and a BSC. The domes are highly fragile and can easily detach. Avoid abrupt plate movement.
    - Avoid dispensing directly onto the gel surface.
25. Return the plate to the incubator.

Step 7. Culture maintenance and media exchange

26. Monitor cultures by brightfield imaging or visual inspection according to the experimental schedule.
27. During media exchange, aspirate and dispense along the well wall. Keep the tip away from the gel construct and microfiber grid.
    - GelControl inserts allow near 100% media exchange without disturbing the domes. Tip plates and position aspirator next to the wall. Aspirate the desired amount of liquid with while reducing the risk of aspirating or damaging the cultures.
    - For media exchange of unsupported domes, be extra vigilant. Aspirate media slowly, and ensure the domes are disturbed as little as possible.
28. Document dome position, deformation, detachment, floating, contraction, and any observations during each exchange.

Step 8. (Optional) Brightfield imaging during culture

29. Monitor your cells during culture using brightfield images.
30. Acquire low-magnification images to document construct position and shape, and higher-magnification images to monitor cellular phenotype.
31. Image supported and conventional cultures with similar settings. The GelControl microfiber grid will be visible in the images.

Step 9. (Optional) Lifting and transfer • Watch Demonstration

32. Use autoclaved forceps to grasp the GelControl handle, not the gel or the microfiber grid.
33. Lift slowly to allow excess medium to drain back into the well. Avoid rapid acceleration or contact with the well wall.
34. Transfer the insert-supported construct into a fresh well, staining vessel, imaging dish, or processing container containing appropriate medium or buffer.
35. After transfer, confirm that the construct remains attached to the insert and centrally positioned. Record any deformation or gel loss.

Step 10. (Optional) alamarBlue metabolic activity assay

36. Prepare alamarBlue working solution according to the reagent manufacturer's protocol and the validated plate-reader settings in your laboratory.
37. Remove or dilute culture medium as required by the assay plan, then add assay solution gently along the well wall. Use 300 µL alamarBlue solution per well in a 24-well format and 1 hour incubation.
38. Include controls containing a clear medium and alamarBlue solution that was incubated without cells.

39. Read fluorescence or absorbance within the linear range of the assay. Normalize within the experiment using appropriate controls.

Step 11. (Optional) Fixation and fluorescence imaging

40. Fix constructs using 4% paraformaldehyde for 30 minutes.
41. Wash gently with PBS. Add and remove liquids along the wall of the well or transfer vessel.
42. Permeabilize constructs using 0.1% Triton X-100 for 30 minutes at room temperature.
43. Stain with antibodies according to the manufacturer's recommendations.
44. Counterstain with Hoechst (2000X) or DAPI and phalloidin for 1 hour at room temperature.
45. Image domes using consistent objective, and processing settings. Note that the microfiber grid may appear in transmitted-light or fluorescence images. When using laser scanning confocal microscope, the grid can be avoided with the desired focal area.

7. Experimental design recommendations

For first-time evaluation, compare GelControl™-supported domes against conventional unsupported domes using the same cells, matrix lot, matrix concentration, dome volume, medium, timepoints, and endpoint assays. The application-note study evaluated two common variables independently: Matrigel concentration and initial cell density.

Design element	Recommended approach	Rationale
Controls	Include conventional unsupported domes prepared with the same Matrigel-cell suspension and volume.	Allows direct assessment of the insert as an independent variable.
Replicates	Use enough biological and technical replicates to quantify variability	GelControl inserts are expected to reduce some experimental variability associated with bulk construct geometry, and mechanical perturbations.
Matrix concentration	Start with a reference established concentration; add lower and higher concentrations.	Organoid cultures are highly dependent on initial conditions
Cell density range	Start with a reference density; add lower, and higher density groups.	Organoid cultures are highly dependent on initial conditions
Primary readouts	Use functional readouts specific to the target cell/tissue properties.	These readouts connect directly to the intended workflow benefits.
Statistics	Report mean ± SD and coefficient of variation, and use a pre-defined test for group comparisons.	SD/CV are particularly useful to evaluate experimental variability and reproducibility.

8. Workflow tips and common pitfalls

Observation	Likely cause	Recommended action
The last several domes seeded are not gelling.	Matrigel begins to gel before seeding is complete. Warm tips, tubes, diluent, or long handling time.	Keep Matrigel and plasticware cold; seed in smaller batches; use cooler racks and ice to keep vials, tips, and diluent cold.
Gel domes are not visible after addition of media	This can be caused be 1) lack of visibility or 2) incomplete gelation. Phenol red diffuses away from gel into the medium, making it difficult to see the outlines of the domes.	Inspect cells on brightfield microscope to determine presence of cells, and inspect gel structure during media exchange.
Bubbles in dome	Fast pipetting or aggressive mixing.	Mix gently and dispense slowly. If minor, most bubbles will fade during culture as cells remodel the matrix.
Gel does not fully fill inner ring	Volume of dispensed liquid and viscosity and surface tension of solution	Validate the appropriate volume of solution to your conditions using acellular trials.

Microfiber grid visible in images	Grid lies beneath the construct and can appear in transmitted light or fluorescence.	This is standard with GelControl. If desired, use focal planes above the grid to minimize appearance in images.
Microfiber grid deformed substantially	The microfiber grid is highly fragile and scraping or push against the grid will cause deformations. The grid can also deform from aggressive pipetting of viscous fluid directly onto it.	Ensure operator avoids directly touching the microfiber grid.

Appendix A. Example calculations for matrix concentration and cell density

Use the equations below to prepare the final Matrigel-cell suspension. Always account for the dilution introduced by cells, medium, drugs, growth factors, or other additives. Prepare slightly more solution than required. Pipetting of the last bit of viscous solutions typically results in bubbles and the most temperature fluctuation.

Output	Equation
Final solution volume	(# of constructs * volume/construct) + overage
Volume of Matrigel stock	(Final conc.* final soln. volume)/(Matrigel stock conc.)
Diluent volume	Final soln. volume – volume of Matrigel stock
Total cells needed	Cell density * final soln. volume

Target Final Values	
Cell Density (cells/mL)	2.00E+06
Final Matrigel Concentration (mg/mL)	6.0
Inputs	
Matrigel stock Concentration (mg/mL)	9.8
#of constructs	12
Solution volume per dome (µL)	60
Outputs	
Final solution volume (µL)	820.00
Volume of Matrigel Stock (µL)	502.04
Diluent volume (µL)	317.96
Total cells needed	1.64E+06

References and resources

1. Malikmammadov E, Tanir TE, Kiziltay A, Hasirci V, Hasirci N. PCL and PCL-based materials in biomedical applications. J Biomater Sci Polym Ed. 2018;29(7–9):863–93. doi:10.1080/09205063.2017.1394711 PubMed PMID: 29053081.

2. Corning Incorporated. Guidelines for Use - Corning Matrigel [Internet]. Corning; [cited 2026 Jul 6]. Available from: https://www.corning.com/catalog/cls/documents/protocols/Matrigel_Matrix_Organoid_Culture_Guidelines_for_Use.pdf

3. Corning Incorporated. Considerations When Optimizing Corning® Matrigel® Matrix Dome Culture [Internet]. Corning; 2025 Sep [cited 2026 Jul 6]. Report CLS-AN-873. Available from: https://www.corning.com/catalog/cls/documents/application-notes/CLS-AN-873.pdf

4. VivoTex. GelControl™ Inserts for Hydrogel Dome Workflows. Application-note.

5. Thermo Fisher Scientific. alamarBlue™ Cell Viability Reagent protocols and user guide.

6. MilliporeSigma. Organoid Culture Frequently Asked Questions.

7. InSphero resources and application notes for 3D spheroid and organoid handling workflows.

GelControl™ is a trademark of VivoTex, Inc.