TheWell BIOSCIENCE

3D Cell Culture and Beyond

VitroGel® EMT

Low Concentration Hydrogel Protocol for Spheroid, Organoid, and Tumoroid Formation

Synthetic, chemically defined hydrogel that supports spheroid formation, tumoroid culture, and uniquely enables EMT biology

Protocol
Catalog #: VHM08, VHM08S

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VitroGel® EMT

Low-concentration hydrogel protocol for spheroid, organoid, and tumoroid formation

Using VitroPrime™ Ultra-Low Attachment, U-Bottom, 96-Well Plate

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A. Introduction

This protocol describes a low-concentration VitroGel® EMT method for forming 3D spheroids, tumoroids, and organoids in the VitroPrime™ Ultra-Low Attachment (ULA), U-Bottom, 96-well plate. By combining a diluted hydrogel scaffold with the gravity-assisted U-bottom geometry, this approach promotes compact cell aggregate formation — even for cell types that do not readily self-aggregate under standard ULA conditions.

Two final gel concentrations are supported:

- 5% VitroGel® EMT — prepared from a 10% intermediate, mixed 1:1 with cells (recommended starting condition)
- 2.5% VitroGel® EMT — prepared from a 5% intermediate, mixed 1:1 with cells (for softer scaffold or faster aggregation)

B. Materials

- **TheWell Bioscience Products**
  - » VitroGel® EMT (Catalog #: VHM08)
  - » VitroPrime™ Ultra-Low Attachment, U-Bottom, 96-Well Plate (Catalog #: VP-ULA96U-8)
  - » RocketCell™ EMT Supplement (50X), XF (Catalog #: RC05-S50)
    (for xeno-free workflows — see Section D)
  - » CytoGrow™ Growth Factors (cell-type specific — see Section G)
  - » Cyto3D® Live-Dead Assay Kit (Catalog #: BM01) (for downstream viability assessment — see Section H)

- **General Lab Supplies**
  - » Sterile 1.5 mL microcentrifuge tubes
  - » Low-retention pipette tips; multichannel or repeater pipette (P200/P300)
  - » Centrifuge (300 × g), CO_2 incubator (37°C / 5%), inverted microscope
  - » Cell culture medium of choice

C. Gel Preparation

1. Allow VitroGel® EMT to equilibrate to room temperature (5–10 min).
2. Select your target final gel concentration (e.g., 2.5% or 5%) for spheroid formation.
3. Prepare the hydrogel intermediate solution at 2X the final concentration as shown in the table below. The solution will be mixed with cells in a 1:1 ratio (refer to Section F).

Target Final Concentration	Intermediate to Prepare	VitroGel® EMT	Complete Medium
5% (recommended)	**10%**	**25 µL**	**225 µL**
2.5%	**5%**	**12.5 µL**	**237.5 µL**

4. Combine VitroGel® EMT and complete medium in a sterile tube at the ratio above.
5. Triturate 4 times with a small-volume pipette, then switch to a P1000 and triturate 10 more times until homogenous.

NOTE: Prepare the gel intermediate fresh before the experiment. Mix with cells immediately before seeding — do not pre-mix in bulk. Replace the FBS-containing medium with your base medium supplemented with RocketCell™ EMT Supplement (50X), XF (Catalog #: RC05-S50) at the recommended 1X final working concentration.

D. Xeno-Free Option

For fully animal-component-free workflows, replace serum-containing medium with a xeno-free alternative throughout this protocol

XENO-FREE OPTION: Replace FBS-containing medium with your base medium supplemented with RocketCell™ EMT Supplement (50X), XF (Catalog #: RC05-S50) at the recommended working concentration.

Use this xeno-free medium in all gel dilution, cell resuspension, and feeding steps.

Pair with the appropriate CytoGrow™ growth factors for your cell type to support proliferation and phenotype maintenance without serum (see Section G).

RocketCell™ Organoid , AOF Essential-Core Medium is also a compatible base medium for organoid applications.

E. Cell Preparation

1. Harvest cells using your standard dissociation method and quench the enzyme in complete medium.
2. Centrifuge at 300 × g for 3 min, aspirate supernatant.
3. Count cells with a cell viability dye such as Trypan Blue.
4. Resuspend at 2X the intended final seeding density in complete medium.

   OPTIONAL: For sensitive cells (e.g., primary cells), add the RocketCell™ Cell Viability Enhancer (1000X) to the medium to achieve a 1X final concentration

F. Cell Seeding

Final seeding volume: 100 µL per well. Prepare 10-20% extra volume to account for dead volume.

1. Gently mix gel intermediate with cell suspension at 1:1 ratio and pipette up and down 8–10 times. Do not vortex. See suggested volumes in the table below.

Component	Per Well	For 96-well plate (+ 10% extra)
2X Cell Stock	50 µL	5.28 mL
Gel Intermediate (10% → 5% final, or 5% → 2.5% final)	50 µL	5.28 mL
Final volume per well	**100 µL**	**9.6 mL**

2. Dispense 100 µL of the mixture per well of the VitroPrime™ ULA, U-Bottom, 96-well Plate using a multichannel or repeater pipette.
3. Optional: Seal the plate with parafilm and centrifuge at 300 × g for 3 min to concentrate cells at the well center.
4. Transfer to the incubator at 37°C / 5% CO_2. Do not disturb the cultures for at least 24 hours.

G. Culture Maintenance

Day 1: Inspect under an inverted microscope. Compact aggregation should be visible within 24 hours for most cell types.

- Medium changes: replace 50% of the medium every 2–3 days by removing it from the well periphery with a micropipette. Dispense fresh warm medium along the well wall.
- For defined or xeno-free culture, supplement medium with CytoGrow™ growth factors appropriate for your cell type (e.g., EGF, FGF-2, HGF, VEGF). CytoGrow™ covers 100+ recombinant proteins across all major growth factor families, validated in cancer, stem cell, and organoid models.

H. Downstream Assays

The following TheWell Bioscience products are optimized for in-well analysis of spheroids formed by this protocol:

Product	Application
Cyto3D® Live-Dead Assay Kit	One-step AO/PI viability staining; optimized for 3D spheroids, tumoroids, and organoids; no pre-mixing; add directly to well and image after 5–15 min at 37°C
VitroGel® Cell Recovery Solution	Non-enzymatic dissociation of spheroids from gel matrix for harvest, replating, or downstream analysis

Appendix: Related Products

Product	Catalog #	Description
VitroGel® GBM EMT Kit, XF	VHM08-K	Complete kit for GBM tumoroid formation with xeno-free method
RocketCell™ EMT Supplement (50X), XF	RC05-S50	Xeno-free serum replacement for spheroid and tumoroid formation
RocketCell™ S1 Supplement (50X), Minus Vitamin A, AOF	RCS1-NA	Animal origin-free B27-like Supplement without vitamin A
RocketCell™ S2 Supplement (100X), AOF	RCS2	Animal origin-free N2-like supplement
RocketCell™ S3 Supplement (50X), AOF	RCS3	Animal origin-free N@-B27 like premixed supplement
RocketCell™ Organoid Essential-Core Medium, AOF	RC04-OCM	Universal animal origin-free base medium; replaces N2/B27 for organoid workflows
VitroPrime™ Ultra-Low Attachment, U-Bottom, 96-Well Plate	VP-ULA96U	Ultra-low attachment plate with U-bottom for spheroid formation
CytoGrow™ Growth Factors	CG-	Over 100+ types of premium recombinant proteins across all major growth factor families
VitroGel® Organoid Recovery Solution	MS04-100	Non-enzymatic cell recovery from VitroGel® matrices and animal-based ECMs
Cyto3D® Live-Dead Assay Kit	BM01	Fast, in-well viability staining for 3D spheroids and organoids

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

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