TheWell BIOSCIENCE

3D Cell Culture and Beyond

VitroGel® Glioblastoma (GBM) Xeno-Free EMT Kit

Ready-to-use kit for 3D GBM tumoroid formation, supporting epithelial-to-mesenchymal transition and enabling long-term tumoroid culture.

Protocol
Catalog #: VHM08-K

Revision No: 1.0
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Developing a glioblastoma multiforme (GBM) tumoroid model with VitroGel® GBM Xeno-Free EMT Kit

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A. Introduction

Highly aggressive tumors, such as GBM, undergo epithelial-to-mesenchymal transition (EMT), a process in which cells shift from an epithelial to a mesenchymal phenotype, allowing extravasation from the primary site, invasion, and metastasis to other organs. To better evaluate EMT and other cancer-associated pathways, it is crucial to develop preclinical models that more accurately mimic the physiological tumor microenvironment. Tumoroids, as three-dimensional (3D) tumor models, have emerged as a powerful platform for preclinical studies due to their ability to recapitulate the tumor microenvironment compared to traditional two-dimensional (2D) models.

The **VitroGel® Glioblastoma (GBM) Xeno-Free EMT Kit** is an excellent tool for 3D GBM tumoroid generation that enables the cells to undergo the epithelial-to-mesenchymal transition for the examination of pathways involved in cancer metastasis, allowing drug screening and automation applications. The product kit features the ready-to-use and synthetic **VitroGel® EMT hydrogel** system in combination with the **RocketCell™ GBM Xeno-Free EMT supplement (50X)** and **VitroPrime™ Ultra-Low Attachment Plate, U-Bottom, 96-Well plate**. The VitroGel® Glioblastoma (GBM) Xeno-Free EMT Kit is designed to induce tumoroid formation from a simple single spheroid within the first two weeks, depending on the cell line used. This tool generates a biologically relevant and well-organized tumoroid model for studying tumor progression, EMT dynamics, and therapeutic response in a preclinical setting.

B. Contents

VitroGel® Glioblastoma (GBM) Xeno-Free EMT Kit (Catalog No. VHM08-K)

Components	Catalog No.	Size
VitroGel® EMT hydrogel	VHM08S	2 mL
RocketCell™ GBM Xeno-Free EMT supplement (50X)	RC05-S50	5 mL
VitroPrime™ Ultra-Low Attachment Plate, U-bottom, 96-well plate	VP-ULA96U	96-well

C. Storage and Stability of VitroGel® Glioblastoma (GBM) Xeno-Free EMT Kit components.

Upon receipt, store the VitroGel® EMT Hydrogel at 4°C for long-term stability.

The RocketCell™ GBM Xeno-Free EMT Supplement (50X) should be stored at –20°C or below. If needed, aliquot the supplement and store the portions at –20°C or below. Avoid repeated freeze–thaw cycles. Once thawed, the supplement remains stable for up to 2 weeks at 4°C.

Storage and Stability:

Product	Temperature	Time
VitroGel® EMT hydrogel	2°C-8°C	1 year
RocketCell™ GBM Xeno-Free EMT supplement (50X)	2°C-8°C; in the dark -80°C to -20°C	2 weeks 1 year
VitroPrime™ Ultra-Low Attachment Plate, U-bottom, 96-well plate	room temperature (25°C)	1 year

Epithelial-to-Mesenchymal Transition (EMT) GBM Tumoroid model

In this protocol, we use U87-MG cells as an example. The basal medium of choice needs to be adjusted based on selected cell type.

MATERIALS

- **VitroGel® Glioblastoma (GBM) Xeno-Free EMT Kit (Catalog No: VHM08-K)**
  - » VitroGel® EMT hydrogel (Catalog No: VHM08)
  - » RocketCell™ GBM Xeno-Free EMT supplement (50X) (Catalog No: RC05-S50)
  - » VitroPrime™ Ultra-Low Attachment Plate, U-bottom, 96-well plate (Catalog No: VP-ULA96U)
- GBM cells (e.g., U87-MG)
- Basal medium of choice (e.g., MEM medium for U87-MG cells)
- Micropipette; low retention pipette tips
- Centrifuge tubes or conical tubes

PROTOCOL

A. Medium preparation

1. Equilibrate the basal medium of choice and RocketCell™ GBM Xeno-Free EMT supplement (50X) to reach room temperature (25°C).

   Note: Avoid freeze/thaw cycles of the RocketCell™ GBM Xeno-Free EMT supplement. If needed, we recommend aliquoting the supplement upon arrival.

2. Prepare complete cell culture medium by combining the basal medium of choice with 1X of the RocketCell™ GBM Xeno-Free EMT supplement.

   Note: The complete cell culture medium is stable for 2 weeks at 4°C.

B. Spheroid Formation

1. Allow the complete cell culture medium with 1X RocketCell™ GBM Xeno-Free EMT supplement to reach room temperature, if prepared in advance.
2. Harvest GBM cells with TrypLE following standard procedures.
3. Prepare cell suspension in complete cell culture medium at a density of 1 x 10^6 cells/mL.
4. Add 20 µL of cell suspension to the wells of the VitroPrime™ Ultra-Low Attachment Plate, U-bottom, 96-well plate.
5. Place the cultureware in the incubator at 37°C overnight to induce spheroid formation.

C. Tumoroid formation from GBM spheroids

1. Equilibrate the VitroGel® EMT hydrogel, RocketCell™ GBM Xeno-Free EMT supplement (50X), and complete cell culture medium to room temperature.
2. Assess spheroid formation with a microscope.
3. Combine VitroGel® EMT hydrogel with the RocketCell™ GBM Xeno-Free EMT supplement (50X) in a 1:1 v/v ratio.

   For example: Mix 500 µL of hydrogel and 500 µL of supplement to have a total volume of 1000 µL mixture.

4. Gently add 40 µL of hydrogel-supplement mixture from step 3 to each well and incubate for 30 minutes at room temperature.

   Important: Position the pipette tip close to the medium in the well when adding the hydrogel mixture to prevent shifting the spheroid from the center. Do not insert the pipette all the way into the well.

5. Add 100 µL of complete cell culture medium on top of the hydrogel and place the cultureware in the incubator at 37°C.
6. Remove and replenish 50% of the medium on top of the hydrogel from the cultures every 2-3 days and monitor the cultures with an inverted microscope to assess tumoroid formation.

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