

# Geltrex™ Flex LDEV-Free, Organoid Qualified, Reduced Growth Factor Basement Membrane Matrix

Catalog Numbers A4000046901, A4000046902, A4000046903

Pub. No. MAN1000813 Rev. A



**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

## Product description

Gibco™ Geltrex™ Flex LDEV-Free, Organoid Qualified, Reduced Growth Factor Basement Membrane Matrix (Geltrex™ Flex Matrix) is a soluble form of basement membrane that is purified from Engelbreth-Holm-Swarm (EHS) tumor cells.

Recommended for the culture of organoids, every lot of Geltrex™ Flex LDEV-Free, Organoid Qualified, Reduced Growth Factor Basement Membrane Matrix is quality control tested for maintaining stable dome form. Mouse intestinal organoids and other organoids are cultured for at least 5 passages in embedded culture to confirm consistent support of organoid culture.

The major components of Geltrex™ Flex Matrix include laminin, collagen IV, entactin, and heparan sulfate proteoglycan. The solution is formulated without phenol red to minimize the potential for estrogen-like effects.

For the culture of primary cells, such as epithelial cells, endothelial cells, smooth muscle cells, angiogenesis assays, neurite outgrowth assays, or bile canaliculi formation assays with polarized hepatocytes, standard Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix can be used. See *Geltrex™ Flex LDEV-Free Reduced Growth Factor Basement Membrane Matrix User Guide* (Pub. No. [MAN1000812](#)).

For the culture of human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC), Geltrex™ Flex LDEV-Free, hESC Qualified, Reduced Growth Factor Basement Membrane Matrix is recommended. See *Geltrex™ Flex LDEV-Free, hESC Qualified, Reduced Growth Factor Basement Membrane Matrix User Guide* (Pub. No. [MAN1000814](#)).

## Contents and storage

Component	Cat. No.	Amount	Storage	Shelf Life <sup>[1]</sup>
Geltrex™ Flex LDEV-Free, Organoid Qualified, Reduced Growth Factor Basement Membrane Matrix	<a href="#">A4000046901</a>	1 mL	-80°C to -20°C	24 months
	<a href="#">A4000046902</a>	5 mL		
	<a href="#">A4000046903</a>	10 mL		

<sup>[1]</sup> Shelf life duration is determined from the date of manufacture.

## Recommended methods for specific applications

Geltrex™ Flex Matrix can be used for various applications that require different basement membrane thicknesses and concentrations. Geltrex™ Flex Matrix comes at lot specific protein concentrations between 9–13 mg/mL.

- To adjust gel stiffness for optimal organoid culture, Geltrex™ Flex Matrix can be diluted to desired final concentration, however note that below 4 mg/mL Geltrex™ Flex Matrix does not form a firm gel.
- For applications where a 3D like environment is desired for the study of cell-cell interactions or complex tissue-like structures, a 3D culture method should be used. See “Cancer organoids in suspension: 3D culture method” on page 3 and “Embedded cultures in domes: 3D culture method” on page 3.

## Procedural guidelines

- Perform all procedures in an aseptic environment, using aseptic techniques, to prevent contamination.
- Source—Murine Engelbreth-Holm-Swarm (EHS) tumor (protein concentration ranges from 9–13 mg/mL). See the Certificate of Analysis for lot-specific information.
- When working with small volumes of Geltrex™ Flex Matrix, dispense the required working volumes, then aliquot and store the remaining solution at -80°C to -20°C in a frost-free freezer.
- Avoid multiple freeze/thaw cycles.
- Geltrex™ Flex Matrix gels in 5–10 minutes when kept above 15°C. Keep Geltrex™ Flex Matrix on ice at all times during handling to prevent premature gelling.
- It is recommended to use Geltrex™ Flex Matrix within a week (7 days) from the time of thawing at 2°C to 8°C.

## Before you begin



1. Thaw Geltrex™ Flex Matrix in a refrigerator overnight at 2°C to 8°C.  
Consider using an aluminum or other cooling block to reduce temperature fluctuations.
2. Mix Geltrex™ Flex Matrix by slowly pipetting up and down. Be careful not to introduce air bubbles.

## Culture method

### Coat the growth surface: 3D sandwich culture method



**Note:** The following procedure is designed for coating an entire 48-well plate. A total of 15 mL of Geltrex™ Flex Matrix is required. To coat fewer or more wells, adjust the volumes accordingly.

1. Culture cells as recommended by the cell supplier to establish a stable population at 37°C in a CO<sub>2</sub> incubator. Growth media, growth factors, serum requirements, and incubation period can vary by cell type.
2. Work on ice and add 250 µL of Geltrex™ Flex Matrix to each well of a sterile 48-well plate.
3. Incubate the plate at 37°C for 30 to 60 minutes to promote gelling of the matrix.
4. Work on ice and add 2 mL of Geltrex™ Flex Matrix to 98 mL of growth medium (2% final concentration) in a sterile container. Label the container "Assay Medium", then swirl to mix.
5. Optional step: When working with very cold-sensitive cells, incubate Assay Medium at 37°C for 5 to 10 minutes in preparation for cell dilution.
6. Harvest cells from culture, then dilute cells in Assay Medium. Generally, cells are diluted 1 × 10<sup>4</sup>–1 × 10<sup>5</sup> cells/mL, depending on the cell type and assay conditions. Optimization may be required.
7. Add 500 µL of cell suspension to each well of the 48-well plate containing Geltrex™ Flex Matrix. Test compounds may also be added at this time.
8. Incubate the plate at 37°C in a CO<sub>2</sub> incubator for 4 days.
9. Observe cell growth and 3D structure formation daily.

- On day 4, carefully remove old medium and replace with new Assay Medium. Repeat on day 8 and day 12.
- When 3D structures have grown to the desired size, harvest cells and analyze.

### Cancer organoids in suspension: 3D culture method



**Note:** The following procedure references the OncoPro™ Tumoroid Culture Medium Kit. To perform this workflow with cancer organoids, the OncoPro™ Tumoroid Culture Medium Kit is recommended but not required.

- Culture cells as recommended by the cell supplier to establish a stable population at 37°C in a CO<sub>2</sub> incubator. Growth media, growth factors, serum requirements, and incubation period may vary by cell type.
- Thaw Geltrex™ Flex Matrix in a refrigerator overnight at 2°C to 8°C.
- Mix Geltrex™ Flex Matrix by slowly pipetting up and down. Be careful not to introduce air bubbles.

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**IMPORTANT!** Be careful not to introduce air bubbles.

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- Recover and seed cancer organoid cultures according to *OncoPro™ Tumoroid Culture Medium Kit User Guide* (Pub. No. [MAN0029372](#)).

**Note:** When plating the initial culture, add ice cold Geltrex™ Flex Matrix into the cell suspension to obtain a final concentration of 2% (volume/volume) basement matrix in medium (example: 100 µL matrix for 5 mL total media volume).

- Add Geltrex™ Flex Matrix to the growing culture according to the *OncoPro™ Tumoroid Culture Medium Kit User Guide* (Pub. No. [MAN0029372](#)).

**Note:** Typically, tumoroids are fed every 2 or 3 days. When feeding the tumoroids, add ice cold Geltrex™ Flex Matrix into the cell suspension to obtain a final concentration of 1% (volume/volume) basement membrane matrix in medium (example: 50 µL matrix for 5 mL total media volume).

- After each culture step, place plate in a 37°C, 5% CO<sub>2</sub> incubator and incubate for 2–3 days.

### Embedded cultures in domes: 3D culture method



- Culture cells as recommended by the cell supplier to establish a stable population at 37°C in a CO<sub>2</sub> incubator. Growth media, growth factors, serum requirements, and incubation period may vary by cell type.
- Thaw Geltrex™ Flex Matrix in a refrigerator overnight at 2°C to 8°C.
- Mix Geltrex™ Flex Matrix by slowly pipetting up and down.

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**IMPORTANT!** Be careful not to introduce air bubbles.

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- Harvest cells from culture and working on ice, resuspend cells in Geltrex™ Flex Matrix.
- Work on ice and pipet 3–4 50 µL drops of cell/ Geltrex™ Flex Matrix onto a well of a 6-well cell culture plate.
- Invert the cell culture plate to form hanging drops of cell/ Geltrex™ Flex Matrix and incubate at 37°C, 5% CO<sub>2</sub> upside-down for 30 minutes to allow cell/ Geltrex™ Flex Matrix polymerization.
- Invert the cell culture plate back to its normal orientation and carefully overlay domes with growth media of choice.
- Place plate in a 37°C, 5% CO<sub>2</sub> incubator for 2–3 days.

## Recommendations for analysis

- Cells can be analyzed in the plate on Geltrex™ Flex Matrix. Alternatively, cells can be carefully transferred to a microscope slide, or embedded in paraffin, then sectioned.
- To fix cells, incubate in 2% formalin in PBS (1X) at room temperature for 20 minutes.
- To fix cells embedded in Geltrex™ Flex Matrix, incubate in 4% formaldehyde plus 1% glutaraldehyde in PBS for 30 minutes to prevent matrix dissolution.

## Related products

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com).

Item	Source
Geltrex™ Flex LDEV-Free Reduced Growth Factor Basement Membrane Matrix (1 mL, 5 mL, 10 mL)	<a href="#">A4000046701</a> , <a href="#">A4000046702</a> , <a href="#">A4000046703</a>
Geltrex™ Flex LDEV-Free, hESC Qualified, Reduced Growth Factor Basement Membrane Matrix (1 mL, 5 mL, 10 mL)	<a href="#">A4000046801</a> , <a href="#">A4000046802</a> , <a href="#">A4000046803</a>
OncoPro™ Tumoroid Culture Medium Kit	<a href="#">A5701201</a>
DMEM/F-12 Medium	<a href="#">11320033</a>
StemPro™ Accutase™ Cell Dissociation Reagent	<a href="#">A1110501</a>
PBS, no calcium no magnesium	<a href="#">14190144</a>
DMEM/F12 + GlutaMAX™-I (1X)	<a href="#">10565042</a>
Penicillin-Streptomycin (10,000 U/mL)	<a href="#">15140122</a>

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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

Revision history: Pub. No. MAN1000813 A

Revision	Date	Description
A	21 March 2025	Initial release.

The information in this guide is subject to change without notice.

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