Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix

Catalog Numbers A1413201 and A1413202

Pub. No. MAN0007332 Rev. 3.0

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**.

Product description

Gibco™ Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix (Geltrex™ Matrix solution) is a soluble form of basement membrane that is purified from Engelbreth-Holm-Swarm (EHS) tumor cells. The solution can be used for promotion and maintenance of a differentiated phenotype in various cell cultures, including primary epithelial cells, endothelial cells, smooth muscle cells, and human induced pluripotent stem cells (iPSCs). Geltrex™ Matrix solution has been used in angiogenesis assays, neurite outgrowth assays, and tumor cell invasion assays.

Geltrex™ Matrix solution gels at 37°C, forming a reconstituted basement membrane that provides the foundation for three-dimensional (3D) culture studies. Basement membranes are continuous sheets of specialized extracellular matrix that form an interface between endothelial, epithelial, muscle, or neuronal cells, and their adjacent stroma. In addition to its role in the physical support and compartmentalization of tissues, basement membrane influences a number of cellular functions, such as proliferation, adhesion, migration, differentiation, and polarization. As a result, basement membrane is implicated in diverse biological processes, including: development, tissue maintenance, regeneration, wound repair, and pathological processes such as, tumor growth and metastasis.

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

Contents and storage

Cat. No.	Amount	Storage	Shelf life[1]
A1413201	1 mL	–80°C to –20°C	36 months
A1413202	5 mL		

[1] Shelf life duration is determined from the date of manufacture.

Recommended methods for specific applications

Geltrex™ Matrix solution can be used for a variety of applications that require different basement membrane thicknesses and concentrations. For differentiation studies of primary cells, we recommend a protein concentration >9 mg/mL. Geltrex™ Matrix solution diluted below 9 mg/mL does not form a gel, and only supports the propagation and maintenance of pluripotency of primary cells, but not their differentiation.

- For applications such as endothelial cell differentiation into capillary-like structures (Tube Assay), a thin gel is needed. See “Coat the growth surface: thin gel method” on page 2.
- For applications such as the differentiation of rat aorta tissue into capillary-like structures (Ring Assay), or cell invasion assays, a thick gel is needed. See “Coat the growth surface: thin gel method” on page 2.
- For applications such as propagation of primary cells that only need a protein layer and not a protein matrix, the thin layer method should be used. See “Coat the growth surface: thin gel method (non-gelling) for propagation of hESC” on page 2.
- 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 “Coat the growth surface: 3D culture method” on page 3.

For more information on 3D cell culture go to **thermofisher.com/3D-cellculture**.

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 12–18 mg/mL). See the Certificate of Analysis for specific lot information.
- When working with small volumes of Geltrex™ Matrix solution, dispense the required working volumes, then store the remaining solution at –80°C to –20°C.
- Avoid multiple freeze/thaw cycles.
- Geltrex™ Matrix solution gels in 5–10 minutes when kept above 15°C. When working from a full 5-mL vial, it is unnecessary to keep the vial on ice if it is used within 5 minutes and the environmental temperature does not exceed 25°C. Because smaller volumes warm more quickly, keep partial tubes and aliquots on ice to prevent premature gelling.

Before you begin

1. Thaw Geltrex™ Matrix in a refrigerator overnight at 2°C to 8°C.
2. Mix Geltrex™ Matrix solution by slowly pipetting up and down. Be careful not to introduce air bubbles.

Coating methods

Coat the growth surface: thin gel method

1. Pipet 50 µL of Geltrex™ Matrix solution per cm^2 onto the growth surface.
2. Place the coated object at 37°C for 30 minutes.

   The coated objects are ready for use.

Coat the growth surface: thick gel method

1. Pipet 150–200 µL of Geltrex™ Matrix solution per cm^2 onto the growth surface.
2. Place the coated object at 37°C for 30 minutes.

   The coated objects are ready for use.

Coat the growth surface: thin layer method (non-gelling)

1. Dilute Geltrex™ Matrix solution to your desired concentration in ice-cold serum-free medium.

   Note: Empirical determination of the optimal coating concentration for your application may be required. A protein concentration of 0.1 mg/mL is a recommended starting concentration for the propagation of primary cells.
2. Add a sufficient amount of the solution to cover the entire growth surface.
3. Place the coated object at 37°C until dry (may take up to 60 minutes).

   The coated objects are ready for use.

Coat the growth surface: thin gel method (non-gelling) for propagation of hESC

1. Dilute 1 mL of Geltrex™ Matrix solution into 99 mL of pre-chilled (4°C) DMEM/F-12 medium (1% final concentration). Determine the optimal coating concentration for your application empirically. If needed, adjust volumes appropriately.
2. Add sufficient diluted Geltrex™ Matrix solution to cover the entire growth surface area.

   For example, add 1.5 mL for a 35-mm dish, or 3 mL for a 60-mm dish.

   The coated dish is stable for two weeks when wrapped with Parafilm™ sealing film and stored at 4°C.

IMPORTANT! Do not allow the coated surface to dry out. It is critical to maintain a storage temperature of 4°C to avoid premature gelling.

3. Incubate the coated plates at 37°C for a minimum of 60 minutes.

4. At the time of use, we recommend keeping the plates at room temperature for one hour before aspirating. Carefully aspirate off the supernatant above the Geltrex™ Matrix coating, then immediately plate cells in the pre-equilibrated cell culture medium.

Coat the growth surface: 3D culture method

Note: The following procedure is designed for coating an entire 48-well plate. A total of 15 mL of Geltrex™ Matrix solution 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_2 incubator. Growth media, growth factors, serum requirements, and incubation period may vary by cell type (as recommended by cell supplier).

2. Working on ice, add 250 µL of Geltrex™ Matrix solution to each well in a sterile 48-well plate.

3. Incubate the plate at 37°C for 30 minutes to promote gelling of the matrix.

4. Working on ice, add 2 mL of Geltrex™ Matrix to 98 mL of growth medium (2% final concentration) in a sterile container. Label the container “Assay Medium", then swirl to mix.

   Any unused Geltrex™ Matrix can be stored at 4°C for up to one week, or in working aliquots at –80°C to –20°C for long-term storage.

5. Incubate Assay Medium at 37°C for 30 minutes in preparation for cell dilution.

6. Harvest cells from culture, then dilute cells in Assay Medium. Generally, cells are diluted 1×10^4–1×10^5 cells/mL, depending upon the cell line and assay conditions. Optimization may be required.

7. Add 500 µL of cell suspension to each well of the 48-well plate containing Geltrex™ Matrix. Test compounds may also be added at this time.

8. Incubate the plate at 37°C in a humidified atmosphere of CO_2 in air for 4 days.

9. Observe cell growth and structure formation daily.

10. On day 4, carefully pipet off old medium using a sterile serological pipet, then replace with new Assay Medium. Repeat on day 8 and day 12.

11. When structures have grown to the desired size, prepare cells for analysis (as recommended by manufacturer), then analyze structures. This step is dependent on the cell line and growth conditions.

Recommendations for analysis

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

Limited product warranty

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Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072
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Revision history: Pub. No. MAN0007332

Revision	Date	Description
3.0	03 November	Updated the shelf life from 18 months to 36 months.
2.00	4 November 2019	• Updated to the current document template, with associated updates to the limited license information, warranty, trademarks, and logos. • Added the following statement to the document: "Manufactured under license by Trevigen, Inc."
1.00	16 January 2014	Baseline for this revision history.