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by life technologies

Geltrex® LDEV-Free Reduced Growth Factor Basement Membrane Matrix

without phenol red

Description

Geltrex® Matrix is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumor cells. The major components of Geltrex® Matrix include laminin, collagen IV, entactin, and heparin sulfate proteoglycan. The extract gels at 37°C forming a reconstituted basement membrane which 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. Basement membrane is thus implicated in diverse biological processes; such as development, tissue maintenance, regeneration, and wound repair; and various pathological processes such as tumor growth and metastasis. Geltrex® Matrix can be used for promotion and maintenance of a differentiated phenotype in a variety of cell cultures including primary epithelial cells, endothelial cells, smooth muscle cells, and human induced pluripotent stem cells (iPSCs). Geltrex® Matrix has been employed in angiogenesis assays, neurite outgrowth assays, and tumor cell invasion assays.

Product	Catalog No.	Amount	Storage	Shelf Life*
Geltrex® LDEV-Free Reduced Growth Factor Basement Membrane Matrix	A1413201 A1413202	1 mL 5 mL	–80°C to –20°C	18 months

*Shelf Life duration is determined from Date of Manufacture.

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Important Information

- **Source:** Murine Engelbreth-Holm-Swarm (EHS) tumor, protein concentration ranges from 12–18 mg/mL. Refer to certificate of analysis for specific lot information.
- Thaw Geltrex® Matrix in a refrigerator at 2°C to 8°C overnight.
- When working with smaller volumes of Geltrex® Matrix, dispense appropriate required working volumes and store at –80°C to –20°C.
- Avoid multiple freeze/thaw cycles.
- Geltrex® Matrix gels in 5–10 minutes above 15°C. When working from a full 5 mL vial, it is unnecessary to keep it on ice if used within 5 minutes and the environmental temperature does not exceed 25°C. Since smaller volumes warm more quickly, partial tubes and aliquots should be kept on ice to prevent premature gelling.
- Formulated without phenol red to minimize potential for estrogen-like effects.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Use

There are many applications for Geltrex® Matrix which require different thicknesses and concentrations. In general, a protein concentration >9 mg/mL is used for differentiation studies of primary cells. Extract diluted below 9 mg/mL does not form a gel, and will only support 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 **Thin Gel Method**.
- 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 **Thick Gel Method**.
- For applications such as propagation of primary cells that only need a protein layer and not a protein matrix; therefore, the thin layer method should be used. See **Thin Gel Method (non-gelling) for Propagation of hESC**.
- For applications where a 3D like environment is desired to study cell-cell interactions or complex tissue-like structures, a 3D culture method should be used. See **3D Culture Method**.

For more information on 3D Cell Culture go to **www.lifetechnologies.com/3D-cellculture**.

Important: It is recommended that the following procedures are performed in an aseptic environment using aseptic techniques to prevent contamination.

Coating Procedures

Thin Gel Method:

1. Thaw Geltrex® Matrix solution. See **Important Information**.
2. Mix Geltrex® Matrix solution by slowly pipetting up and down; be careful not to introduce air bubbles.
3. Pipet 50 µL per cm^2 onto the growth surface.
4. Place coated object at 37°C for 30 minutes.
5. Coated objects are ready for use.

Thick Gel Method:

1. Thaw Geltrex® Matrix solution. See **Important Information**.
2. Mix Geltrex® Matrix solution by slowly pipetting up and down; be careful not to introduce air bubbles.
3. Pipet 150–200 µL per cm^2 onto the growth surface.
4. Place coated object at 37°C for 30 minutes.
5. Coated objects are ready for use.

Thin Layer Method (non-gelling)

1. Thaw Geltrex® Matrix solution. See **Important Information**.
2. Mix Geltrex® Matrix solution by slowly pipetting up and down; be careful not to introduce air bubbles.
3. Dilute the extract to your desired concentration in ice-cold serum-free medium. 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.
4. Add a sufficient amount of solution to cover the entire growth surface.
5. Place coated object at 37°C until dry (may take up to 60 minutes).
6. Coated objects are ready for use.

Thin Gel Method (non-gelling) for Propagation of hESC

1. Thaw Geltrex® Matrix solution. See **Important Information**.
2. Mix Geltrex® Matrix solution by slowly pipetting up and down; be careful not to introduce air bubbles.
3. Dilute 1 mL Geltrex® Matrix solution into 99 mL pre-chilled (4°C) DMEM/F-12 medium (1% final concentration). Determine optimal coating concentration for your application empirically. Adjust volumes accordingly.
4. Add sufficient diluted Geltrex® Matrix solution to cover the entire growth surface area (e.g., 1.5 mL for 35-mm dish, 3 mL for 60-mm dish). The coated dish is stable for two weeks when wrapped with Parafilm® sealing film and stored at 4°C. *Do not allow coated surface to dry out. It is critical to maintain a storage temperature of 4°C to avoid premature gelling.*
5. Incubate coated plates at 37°C for a minimum of 60 minutes.
6. At time of use, *we recommend keeping plates at room temperature for one hour before aspirating.* Carefully aspirate off the supernatant above the Geltrex® coating and immediately plate cells in pre-equilibrated cell culture medium.

3D Culture Method

Note: To perform this procedure using every well of a 48-well plate, a total of 15 mL of Geltrex® Matrix solution is required. Volumes and number of wells used can be adjusted accordingly.

1. Culture cells as recommended by cell supplier to establish a stable population at 37°C in CO_2 incubator. Growth media, growth factors, serum requirements, and incubation period may vary by cell type (as recommended by cell supplier).
2. Thaw Geltrex® Matrix solution. See **Important Information**.
3. Working on ice, add 250 µL of Geltrex® Matrix solution to each well in a sterile 48-well plate; incubate plate at 37°C for 30 minutes to promote gelling of matrix.
4. Working on ice, add 2 mL Geltrex® Matrix to 98 mL of growth medium (2% final concentration) in a sterile container, label container "Assay Medium," and swirl to mix. Volumes can be adjusted accordingly. Any unused Geltrex® Matrix can be stored at 4°C up to one week or stored in working aliquots at –80°C to –20°C.
5. Incubate Assay Medium at 37°C for 30 minutes in preparation for cell dilution.
6. Harvest cells from culture, and dilute cells in Assay Medium. Generally, cells are diluted to between 1×10^4 to 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 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, and replace with new Assay Medium. Repeat on day 8 and day 12.
11. When structures have grown to desired size, prepare cells for analysis (as recommended by manufacturer), and analyze structures. This step is dependent on cell line and growth conditions.

Recommendations for analysis

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

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

			
Use By:	Manufacturer	Batch code	Catalog number
			
Temperature Limitation	Consult instructions for use	Caution, consult accompanying documents	Sterilized using aseptic processing techniques

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email **techsupport@lifetech.com**