

Geltrex[™] LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix

Catalog Numbers A1413301 and A1413302

Pub. No. MAN0007336 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

Geltrex[™] LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix (Geltrex[™] Matrix solution) is used routinely for attachment and maintenance of human embryonic stem cells (hESCs). The solution can also 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 to form a reconstituted basement membrane. 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. Each lot of Geltrex[™] Matrix solution has been function-tested to provide the unique micro-environmental requirements for the growth and maintenance of pluripotency of hESCs, therefore eliminating the need to test multiple lots.

Contents and storage

| Cat. No. | Amount | Storage | Shelf life ^[1] |
|----------|--------|----------------|---------------------------|
| A1413301 | 1 mL | -80°C to −20°C | OC months |
| A1413302 | 5 mL | | 36 months |

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

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

Geltrex[™] Matrix solution is tested for hESC applications. For differentiation studies of hESCs, 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 hESCs when grown with media designed for feeder-free propagation of hESCs.

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

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.

Lot qualification

12-well cell culture plates were coated with each test lot of Geltrex[™] LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix. Human ESCs were grown on coated control and test plates in StemPro[™] hESC SFM, then monitored for expansion. A hESC negative control was grown with retinoic acid to induce differentiation and was used as an internal PCR control (See Figure 1).

After completion of the hESC Growth Assay, the test and control samples were assessed by PCR analysis (See Figure 2).

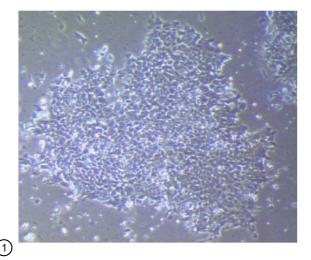
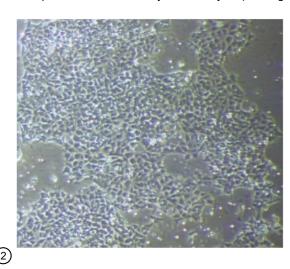


Figure 1 Growth Assay

1 Human ESC control



② Human ESC negative control

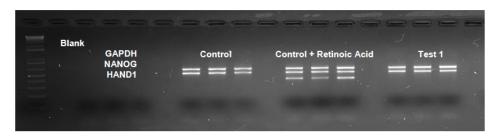


Figure 2 PCR analysis

Related products

| Product | Cat. No. | | |
|---|-----------|--|--|
| DMEM/F-12, GlutaMAX™ Supplement | 10565 | | |
| 2-Mercaptoethanol | 21985023 | | |
| FGF-Basic (AA 10-155) Recombinant Human Protein | PHG0026 | | |
| StemPro™ hESC SFM | A1000701 | | |
| StemPro™ MSC SFM | A10332-01 | | |
| StemPro™ MSC SFM XenoFree | A10675-01 | | |
| StemPro™ EZPassage™ Disposable Stem Cell Passaging Tool | 23181010 | | |
| CTS™ TrypLE™ Select Enzyme | A1285901 | | |
| KnockOut™ DMEM | 10829018 | | |
| CTS™ KnockOut™ SR XenoFree Kit | A1099201 | | |
| KnockOut™ DMEM/F-12 | 12660012 | | |
| CTS™ GlutaMAX™-I Supplement | A1286001 | | |
| Human Umbilical Vein Endothelial Cells (HUVEC) | C0035C | | |

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Revision history: Pub. No. MAN0007336

| Revision | Date | Description |
|----------|------------------|--|
| 3.0 | 03 November 2020 | Updated the shelf life from 12 months to 36 months. |
| 2.00 | 5 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." Corrected the product shelf life from 18 months to 12 months. |
| 1.00 | 17 January 2014 | Baseline for this revision history. |

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