ISO9001 & ISO13485 & ISO11137

# GelNest™ Matrix, LDEV-Free

**Gel Concentration Inquiry: If the label on the bottle is lost, please click or copy the link below to your browser, and select "Related Reference Tools-> Certificates" to download the batch-specific COA.**

https://www.nestscientificusa.com/product/detail/636882319776419840

## Product overview

GelNest™ Matrix is prepared from basement membrane components extracted from mouse tumor tissues. The main components include laminin, type IV collagen , heparan sulfate proteoglycan, etc. These components can provide the support and signals required for cell adhesion, differentiation, and proliferation. They can also simulate the characteristics of the basement membrane in a physiological environment and improve the success rate and effect of cell culture.

In addition to basement membrane components, GelNest™ Matrix is also rich in a variety of growth factors. These growth factors can promote cell differentiation, proliferation, and migration, further mimicking cell signaling pathways and interactions in physiological environments. GelNest™ Matrix has a wide range of application prospects, especially in tissue engineering, cell culture and research. It can be used for research on organoid culture, stem cell differentiation, angiogenesis, migration or invasion, and *in vivo* tumorigenesis.

## Product information

| Product number | Product name | Packaging specifications |
|---|---|---|
| 211212 | GelNest™ Matrix, LDEV-Free | Bag Package, 5 mL/bottle, 1 bottle/bag |
| 211222 | GelNest™ Matrix, Phenol Red-Free, LDEV-Free | Bag Package, 5 mL/bottle, 1 bottle/bag |
| 211211 | GelNest™ Matrix, LDEV-Free | Bag Package, 5 mL/bottle, 2 bottle/bag |
| 211221 | GelNest™ Matrix, Phenol Red- | Bag Package, 5 mL/bottle, 2 bottle/bag |

| | | |
|---|---|---|
| | Free, LDEV-Free | |
| 211312 | GelNest™ Matrix, LDEV-Free, Ultra-low Endotoxin | Bag Package, 5 mL/bottle, 1 bottle/bag |
| 211322 | GelNest™ Matrix, Phenol Red-Free, LDEV-Free, Ultra-low Endotoxin | Bag Package, 5 mL/bottle, 1 bottle/bag |

## Product parameters

| | |
|---|---|
| **Source** | Mouse tumor tissue basement membrane components |
| **Formulation*** | Standard Formulation |
| **Protein concentration** | See label, or please download the COA from our official website to obtain a lot-specific concentration. |
| **Appearance** | GelNest™ Matrix is liquid at 4°C but forms a gel at 37°C. Phenol red-containing gel appears bright yellow when frozen, and red at temperatures above 0° C. |
| **Applications** | Suitable for 2D primary cell culture, organoid construction, culture, differentiation, invasion assays, *in vitro* angiogenesis experiments. |
| **Storage and shelf life** | Store in a refrigerator at -20°C (frost-free function off) or a -80°C freezer for up to 2 years. It is recommended to aliquot the thawed product into single-use portions and store it in -20°C or -80°C for up to 2 years. |
| **Precautions** | GelNest™ Matrix will start to solidify when the temperature is higher than 10°C. Please try to operate on ice as much as possible, and it is recommended to pre-cool the consumables that directly contact the gel, such as pipette tips. |

*Please use phenol red-free matrix gel for colorimetric analysis. For the preparation of a more defined basement membrane coating, it is recommended to use growth factor reduced matrix gel.

## Experimental procedures

**GelNest™ Matrix Handling Recommendations**

| Application Type | Dilution Factor | Final Concentration | Volume Added | Experiments |
|---|---|---|---|---|
| Thin Gel Coating | 1:10 | >1mg/mL | 50μL/cm$^2$ | 2D primary cell culture |
| | 1:50 | >0.1mg/mL | | Invasion assay with cell culture inserts |
| | 1:100 | >0.01mg/mL | 300μL/cm$^2$ | Stem cell culture |
| Thick Gel Coating | No Dilution | >10mg/mL | 150-200μL/cm$^2$ | Angiogenesis/tube formation |
| Thick Gel Embedded | Gel : Cell Mixture<7:3 | >7mg/mL | | Organoid culture |
| | Gel : Cell Mixture ≈ 1:1 | >18mg/mL | 200μL/sample | In vivo tumorigenesis assay |

Please determine the specific experimental steps based on cell types, culture conditions, and application experience.

## Organoid culture

**1.** Re-suspend the single cell suspension used for organoid culture in pre-cooled basal medium at 4°C, and count the cells.

**2.** Mix GelNest™ Matrix with the cells (recommended dilution ratio should be no less than 70%) and add the mixture to a preheated 24-well plate, each well containing approximately 5x10$^4$ cells and 60μL of the matrix gel.

**3.** Immediately place the well plate into the incubator. After about 10 minutes, the matrix gel will solidify.

**4.** Add 500μL of organoid culture medium for culture.

**5.** Wait 3-5 days for the organoids to form. Finally, the sensitivity of organoids to various drugs can be determined by imaging live cells through high-content microscopy.

* It is recommended to use GelNest™ Matrix, for Organoid Culture (NEST 211282) for better

results.

## Angiogenesis experiments

**1.** Replace complete culture medium with starvation medium: DMEM medium containing 0.2% FBS, 2mM L-glutamine, 1mM sodium pyruvate, 100U/mL penicillin and 100μg/mL streptomycin. Starve the cells for 24 hours.

**2.** Spread 50μL GelNest™ Matrix(no dilution recommended) evenly on the bottom of a 96-well plate. *(To prevent the matrix gel from adhering to the inner wall of the pipette head, you can use the pipette head to blow FBS once before absorbing the matrix gel, and rinse the inner wall of the pipette head with FBS.)*

**3.** Place the 96-well plate in a 37°C cell culture incubator for 30 minutes to solidify the matrix gel.

**4.** Digest HUVEC cells and perform cell counting.

**5.** Seed $5x10^4$ HUVEC cells to a 96-well plate containing the matrix gel for a total of 200 μL for each well. Place the 96-well plate into the incubator for culture.

**6.** The vascular-like network structure will form within 3 to 12 hours. This is the best time to observe.

**7.** At the optimal observation time point, remove the medium carefully and stain the cells with Calcein AM (green) medium at a concentration of 1/1000. Use a microscope to image the cells and record the morphology and characteristics of the vascular network.

* It is recommended to use GelNest™ Matrix, for Angiogenesis Experiment, Low Endotoxin (NEST 211492) for better results.

## Invasion experiment

**1.** Use HT-1080 cells in MEM medium supplemented with 10% fetal bovine serum and culture them to a cell confluence of 80% to 90% before use.

**2.** Take 20μL of GelNest™ Matrix, dilute it to 1000μL with serum-free MEM ( 1:50 dilution).

**3.** Gently pipette up and down to thoroughly mix the gel.

**4.** Add 100 µL of the diluted matrix gel mixture to the center of each cell culture insert (NEST transwell product) so that the matrix gel mixture evenly covers the surface of the insert. Incubate the culture dish at 37°C for 1 hour to allow gel formation.

**5.** After trypsinization of the cells (typically, for a 6-well plate, digest the cells with 200µL of trypsin at 37°C for 3 minutes, then terminate the digestion with 10% serum, centrifuge at 300xg for 3 minutes), resuspend the cells in serum-free MEM culture medium.

**6.** Count cells and take 750µL of cells at a starting density of $1x10^6$/mL (expected to use 10 wells with $7.5x10^4$ cells per well, a total of 750,000 cells), and dilute it with MEM serum-free medium to 1.5mL.

**7.** Seed 150 µL of cell suspension into the upper chamber of each cell culture insert, resulting in $7.5x10^4$ cells/well.

**8.** In the experimental group, add 800 µL of culture medium containing 10% FBS as a chemoattractant to the lower chamber, while in the control group, add 800 µL of serum-free culture medium to the lower chamber. Incubate the cells overnight at 37°C with 5% carbon dioxide in a humidified incubator.

**9.** Discard the supernatant medium from the cell culture insert and wash twice with PBS. Stain the cells on the underside of the membrane with crystal violet for 10 minutes.

**10.** Wash the cell culture inserts twice with PBS to remove unbound crystal violet. Use a moist cotton swab to gently remove the cells from inside the cell culture insert, then air-dry.

**11.** Observe and capture images of the invaded cells under a microscope.

**12.** Dilute acetic acid to 33% (v/v) with ddH2O to elute the bound crystal violet. Add 400µL 33% acetic acid to each cell culture insert and shake on a shaker for 10 minutes. Transfer the eluate from the lower chamber to a 96-well transparent microplate, and measure the absorbance at 590 nm using a microplate reader.

## Safety recommendations and limitations

Please follow good laboratory safety practices.

**For research use only. Not intended for diagnostic or therapeutic purposes. Contains ingredients of animal origin.**

## Technical support and contact information

For FAQ, GelNest™ Matrix Selection Guide, Quality Assurance COA/COC or other technical support and product issues, please refer to our website or use the following contact information.

Production and after-sales service unit: Wuxi NEST Biotechnology Co., Ltd.

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