ISO9001 & ISO13485 & ISO11137

GelNest™ Matrix, Phenol Red-Free, LDEV-Free

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
211222	GelNest™ Matrix, Phenol Red-Free, LDEV-Free	Bag Package, 5 mL/bottle, 1 bottle/bag

Product parameters

Source	Mouse tumor tissue basement membrane components
Formulation*	Without phenol red
Protein concentration	Please refer to the COA/COC for batch-specific protein concentration.
Appearance	GelNest™ Matrix is liquid at 4°C but forms a gel at 37°C.
Applications	This product is suitable for organoid construction, culture, differentiation, and *in vitro* and *in vivo* angiogenesis experiments.
Storage and shelf life	It is recommended to aliquot the melted product into single-use portions

	and store it in a -80°C freezer. The product has a shelf life of 2 years.
Precautions	GelNest™ Matrix will start to solidify when the temperature is higher than 10°C. Please operate on ice.

*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

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 the cells with GelNest™ Matrix solution 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 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.

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). Gently pipette up and down to thoroughly mix the matrix gel. Next, 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.
3. 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. 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. Then, seed 150 µL of cell suspension into the upper chamber of each cell culture insert, resulting in $7.5x10^4$ cells/well. 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.

4. 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, and then 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. Observe and capture images of the invaded cells under a microscope.
5. 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.

Feeder-free culture of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs)

1. Take the GelNest™ Matrix from frozen storage and thaw in an ice bath at 4°C overnight. Use a pre-cooled pipette tip to slowly pipette the matrix gel 3 times to mix. Use pre-cooled pipette tips to aliquot the thawed matrix gel. If bubbles form, briefly centrifuge the aliquoted matrix gel using a handheld centrifuge to remove bubbles.
2. Place the cell culture plate in the 37°C incubator to preheat.
3. Dilute the matrix gel solution at a ratio of 1:100 with serum-free medium that is pre-cooled at 4°C, and completely cover the culture plate with the matrix gel diluent. It is recommended to use 300µL/cm^2 of matrix gel diluent in a culture dish.
4. Allow the culture plate containing the diluted matrix gel to sit at room temperature for 1 hour.
5. Remove the remaining matrix gel diluent and immediately seed the stem cells with premixed mTeSR solution onto the culture plate. Be careful not to let the modified culture plate surface dry out.

* It is recommended to use GelNest™ Matrix, for hESC Culture (NEST 211272) for better results.

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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