1. What experiments is high-concentration GelNest matrix used for?

The GelNest high-concentration matrix is suitable for *in vivo* application studies, such as tumor growth promotion. The high protein concentration can also help the GelNest matrix remain intact after being injected subcutaneously into mice, which is beneficial for the *in situ* maintenance of injected tumor cells and/or angiogenic factors, making it suitable for *in situ* analysis and/or subsequent removal.

2. How to use GelNest matrix for 3D culture? How to make a 3D gel? Do cells need to be embedded in the GelNest matrix?

The GelNest high-concentration matrix can be used for *in vivo* application studies, such as preparing a thick layer for 3D cell culture. Cells can be embedded in the GelNest matrix or inoculated on the surface of the GelNest matrix (covering method).

3. When using GelNest matrix, do you need to pre-cool the pipette tips and centrifuge tubes?

Yes. Because the GelNest matrix will start to gel at temperatures above 10℃, we recommend using pre-cooled pipettes, tips, and centrifuge tubes when operating the matrix.

4. Does GelNest matrix polymerize quickly?

The GelNest matrix will quickly polymerize into a gel at temperatures between 10℃ and 35℃.

5. In what situations do you need to use phenol red-free GelNest matrix?

For experiments involving color detection, it is recommended to use phenol red-free GelNest matrix, such as using fluorescent dyes or Drabkins method to count endothelial cell tube formation experiments. For endometrial cell culture, phenol red-free GelNest matrix should also be used.

In addition, phenol red has a similar structure to non-steroid estrogen and has estrogen-like effects. It may have the ability to interfere with endocrine and hormone metabolism in experimental animals.

6. How to harvest cells from GelNest matrix?

It is recommended to use neutral protease or cell recovery solution to harvest cells cultured in the GelNest matrix. Compared with trypsin, collagenase or other protein hydrolysis enzymes, neutral protease can obtain single cell suspension more gently and effectively, without damaging cells or cell surface proteins. For cells that need to continue to be inoculated or tested, the use of neutral protease will not cause damage. In addition, neutral protease can also be used for tissue separation.

For metabolic studies and RNA extraction, it is recommended to use the cell recovery solution for non-enzymatic cell collection at 4℃. Because the GelNest matrix contains trace amounts of RNA, when performing RNA analysis, a control group of GelNest matrix (without inoculation of cells) should be set. Other methods for harvesting cells from the GelNest matrix: lower the temperature to 4℃-6℃ to disassemble the GelNest matrix, which takes some time and is only suitable for part of the application.

7. How long can the culture dishes coated with GelNest matrix be stored?

The coated culture dishes are best used on the same day, and the specific situation depends on the

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experimental purpose. If you need to save, you can store it in a 37°C incubator for up to 7 days. During storage, the surface of the GelNest matrix needs to be evenly covered with serum-free medium to keep it moist.

8. In what situations should thin gel be chosen? When should thick gel be used? What are the applications of 3D culture?

Thin gel is mainly used to assist cell attachment, which is beneficial for cell proliferation. For example, for primary cell culture, a thin layer of protein is needed for assistance, so thin gel can be chosen; thick gel is mainly used for 3D cell culture, such as rat aorta tissue differentiation into capillary-like structures (RingAssay), and cell invasion experiments, etc.; 3D cell culture experiments are mainly used to study the interaction between cells and complex structures such as biological tissues.

9. What concentration of GelNest matrix should be chosen for the angiogenesis (endothelial tube formation) experiment?

For this experiment, the minimum concentration of GelNest matrix should not be less than 10mg/mL.

10. What is the minimum gel concentration of GelNest matrix used in thick gel experiments?

Different experimental purposes require different concentrations of GelNest matrix, and users should determine according to the specific experimental needs. The minimum gel concentration of GelNest matrix for thick gels is 7mg/mL. Don't simply dilute by volume ratio when diluting, as the concentration of GelNest matrix varies between batches. It should be calculated based on the final working concentration (mg/mL) to determine the amount of diluent (such as PBS or serum-free medium) to be added. For GelNest matrix used for in vivo research, in order to avoid incomplete gel formation, the final working concentration should not be less than 10mg/mL.

11. How long can the matrix gel block of GelNest matrix be maintained in the body?

The matrix gel block can be maintained in the body for at least one week.

12. How to dilute GelNest matrix?

Use pre-cooled serum-free medium or PH7.4 PBS on ice.

13. How should the GelNest matrix be pipetted?

It is recommended to use pre-cooled pipettes or syringes, and the pipettes and tips also need to be pre-cooled. Avoid touching the bottom of the bottle when aspirating; avoid being too fast or too forceful when dispensing. If a pipette (Pipets) is used, when you need to dispense 5mL, you should draw up 6mL, and stop when there is still 1mL in the pipette to obtain better accuracy; if an automatic pipette (Pipetman) is used, press to the second stage to aspirate, then press to the first stage to dispense.

14. Why is my GelNest matrix very viscous?

The higher the protein concentration of the matrix gel, the more viscous the gel. If the concentration is

higher than 13.0mg/mL, the matrix gel will appear very heavy. The GelNest matrix products are relatively viscous before dilution. The viscous high-concentration GelNest matrix can be used directly without dilution, such as for culturing tumor cells and/or angiogenic factors. After being injected into mice, the cells can be kept *in situ*, which is convenient for *in situ* analysis and/or subsequent removal; or after dilution, it can be used according to the usage method of the standard concentration GelNest matrix product, and the specific dilution concentration is determined according to the experimental requirements. In addition to being viscous due to the high concentration of the product itself, the state of the matrix gel is also related to the changes in temperature during transportation and storage conditions. The whole transportation process must be refrigerated with dry ice. If the refrigerator storing GelNest matrix has an automatic defrost function, the refrigerator will heat up during the defrosting process, which may cause the matrix gel to gel. So, never store GelNest matrix in such refrigerators. In order to ensure the use effect of GelNest matrix, the number of freeze-thaw cycles should be minimized. When you get a new GelNest matrix, please divide it according to a single dose. Each time the GelNest matrix is thawed, it should be placed on ice.

15. Why does GelNest matrix gel at 37°C, but is liquid at 4°C?

GelNest matrix is a recombinant basement membrane extracted from mouse tumors. The freshly extracted raw materials mainly include the following components: laminin, type IV collagen, nidogen, basement membrane polysaccharide, epidermal growth factor, insulin-like growth factor, and other growth factors. These proteins constitute the basic structure of GelNest matrix. Under temperature conditions of 22℃-37℃, the covalent bonds between macromolecules can bind, causing GelNest matrix to form a gel. However, under low-temperature conditions (such as 4℃), there is not enough energy to cause covalent bonds to bind, so GelNest matrix is in a liquid state.

16. Can GelNest matrix be repeatedly freeze-thawed?

It is recommended that users aliquot the gel into single-use quantities after the first thawing and then store it.

17. Why didn't the cells attach? Did the GelNest matrix also fall off?

First, it is necessary to check whether the cell inoculation concentration is too high. The amount of GelNest matrix should be the same as the amount of culture medium in the cell culture system. If the GelNest matrix is diluted to a too low concentration, the formed gel is easy to separate from the surface of the tissue culture vessel.

18. How should the precipitate that appears in the undiluted GelNest matrix be handled?

Centrifuge at low speed at 4℃ to remove the precipitate.

19. How should the unused GelNest matrix be stored?

It is not recommended to keep and reuse GelNest matrix that has been mixed with cell culture medium or buffer but has not been used up.

20. Can GelNest matrix be stored at -70℃?

Yes. GelNest matrix can be stored at -70℃. It is recommended that customers aliquot the entire bottle of GelNest matrix and store it in small tubes made of polypropylene or other materials that can withstand ultra-low temperature conditions for easy storage and use.

21. Does GelNest matrix have self-fluorescence?

GelNest matrix is a protein mixture, so the components of GelNest matrix that may cause fluorescence are protein components. If you need to use fluorescence to monitor cell growth status, it is recommended that the user establish a control experiment and compare under the required wavelength conditions to eliminate background fluorescence.

22. If cells cultured with GelNest matrix need to be sectioned or immunohistochemistry and immunofluorescence tests, how should they be fixed? How to avoid disintegration?

You can use 2% paraformaldehyde for fixation. To avoid disintegration after fixation, 1% glutaraldehyde can be added. Glutaraldehyde is commonly used as a fixative for electron microscopy observation. If users need to perform immunofluorescence tests, there will be obvious background fluorescence after adding glutaraldehyde. To solve this problem, we suggest that users use $NaBH_4$ for quenching after fixation. $NaBH_4$ is prone to produce bubbles, and this step must be carefully operated on a horizontal operation platform to avoid shaking and minimize the formation of bubbles. In addition, users can also try to use a lower concentration of glutaraldehyde for fixation, such as 0.1% to 0.5%, the lower the concentration, the less background fluorescence signal.

23. Why is the color of GelNest different?

Due to the reaction of carbon dioxide and bicarbonate buffer and phenol red, GelNest that is frozen or just thawed may show different colors, ranging from straw yellow to dark red.

Phenol red appears bright yellow when frozen or in an acidic environment, and red when approaching physiological pH or above 0ºC.

The appearance of color differences in GelNest is normal, will not affect product quality, and will return to a uniform color after balancing with 5% carbon dioxide.

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