GelNest™ Matrix User Tips

How to thaw?

1. GelNest™ matrix is liquid at 4℃ and forms a gel state at 37℃. Gelation starts above 10℃.
2. Embed a vial of GelNest™ matrix on ice and place it in a 4℃ refrigerator. Wait for it to thaw completely.

How to dilute?

1. Pre-cool the pipette tips, culture medium, and laboratory vessels that come into contact with the GelNest™ matrix gel.
2. Dilute the thawed GelNest™ matrix gel with ice-cold serum-free culture medium.
3. Mix thoroughly by pipetting up and down on the ice or gently rotating.
4. The dilution ratio depends on the protein concentration, not the standard volume.

Storage and operational precautions:

1. Before aliquoting, GelNest™ matrix gel can be stored in a -20℃ freezer or a -80℃ ultra-low temperature freezer.
2. For the first use, aliquot after thawing according to the single-use amount and store in a -80℃ or -20℃ freezer. The shelf life is 2 years. It is not recommended to store thawed/diluted GelNest™ matrix gel for a long time.
3. Do not store the product in a frost-free freezer, on the refrigerator door, or in a frequently opened refrigerator.
4. Pre-cool the pipette tips, microcentrifuge tubes, etc., and preferably perform operations on ice.

Related Experimental Flows

1. Coating culture experiment:

   2D culture: Dilute the gel (dilute the coated gel at a ratio of 100:1).

   No dilution: Thin coating: 50μL/cm2, thick coating: 150-200μL/cm2

   a) Mix the matrix gel evenly.
   b) Add non-diluted or diluted gel solution proportionally into a 6-well plate/24-well plate.
   c) Incubate at 37°C for 30 minutes to allow gel formation. Optionally, remove excess liquid, then seed cells and add culture medium for cultivation.
   d) d) Observe cell status.
2. Tumor invasion experiment:

   When coating the gel, do not place cells on top. Seed tumor cells after coating.

   a) Coat the matrix gel on the surface of the culture chamber membrane.
   b) Seed tumor cells (HT-1080) in the culture chamber (on top of the gel).
   c) Add chemoattractants (such as serum, etc.) in the recipient chamber and culture overnight.
   d) Use a cotton swab to scrape off non-invading cells from the top. Dye and observe.
3. Stem cell culture experiment:

   a) Dilute the specialized gel for stem cells (~1:100) and coat it onto a 6-well plate.
   b) Seed stem cells and culture them.

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c) Change the medium daily and passage the cells weekly to maintain the pluripotency of the cells.

4. *In vitro* angiogenesis assay
   a) Coat a gel with a concentration greater than or equal to 10mg/mL (or dilute to 10mg/mL) onto a 24-well plate.
   b) Incubate the gel at 37 ℃ to form a gel.
   c) Seed HUVEC/HMVEC/HMEC cells with a confluence of 70-80% onto the coated gel in the 24-well plate.
   d) Culture for 6-12 hours. Observe.

5. Organoid culture experiment

Primary differentiation:

   a) Obtain primary tissue.
   b) Dissociate the cells.
   c) Mix the primary cells with matrix gel, and seed for expansion.

iPSC induction:

   a) Extract peripheral blood mononuclear cells (PBMCs) or skin fibroblasts.
   b) Reprogram the cells into iPSCs.
   c) Induce directed differentiation using different inducing factors, mix with matrix gel after formation of organoid-like structures, and continue culturing.
   d) Observe the formed organoids.

6. *In vivo* tumor formation
   a) Mix high concentration gel with tumor cells.
   b) Inject the mixed gel subcutaneously into mice using a large-gauge needle (21-25G).
   c) Culture for a period of time.
   d) Analyze tumor formation using different methods:
      i. Morphology and size.
      ii. Tissue sections.
      iii. Analysis of angiogenesis.