Corning® Matrigel® Matrix

Frequently Asked Questions

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1. **What is Corning Matrigel matrix?**

   Corning Matrigel matrix is a reconstituted basement membrane preparation that is extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma, a tumor rich in extracellular matrix proteins. This material, once isolated, is approximately 60% laminin, 30% collagen IV, and 8% entactin. Entactin is a bridging molecule that interacts with laminin and collagen IV and contributes to the structural organization of these extracellular matrix molecules. Corning Matrigel matrix also contains heparan sulfate proteoglycan (perlecan), TGF-β, epidermal growth factor, insulin-like growth factor, fibroblast growth factor, tissue plasminogen activator, and other growth factors which occur naturally in the EHS tumor. There are also residual matrix metalloproteinases derived from the tumor cells.

2. **What is the growth factor (GF) concentration in Corning Matrigel matrix?**

   Amounts of Growth Factors (GF) Present in Corning Matrigel Matrix vs. Growth Factor Reduced (GFR) Corning Matrigel Matrix

Growth Factor	Range of GF Concentration in Corning Matrigel Matrix	Average GF Concentration in Corning Matrigel Matrix	Typical GF Concentration in GFR Corning Matrigel Matrix
EGF	0.5 - 1.3 ng/mL	0.7 ng/mL	<0.5 ng/mL
bFGF	<0.1 - 0.2 pg/mL	n.a.*	n.d.**
NGF	<0.2 ng/mL	n.a.*	<0.2 ng/mL
PDGF	5 - 48 pg/mL	12 pg/mL	<5 pg/mL
IGF-1	11 - 24 ng/mL	16 ng/mL	5 ng/mL
TGF-β	1.7 - 4.7 ng/mL	2.3 ng/mL	1.7 ng/mL

   *n.a. = not applicable
   **n.d. = not determined

3. **Why is my Corning Matrigel matrix different colors?**

   Color variations may occur in frozen or thawed vials of Corning Matrigel matrix ranging from straw yellow to dark red due to the interaction of carbon dioxide with the bicarbonate buffer and phenol red. Phenol red is bright yellow below -20°C or acidic pH, inactive between -20°C and 0°C, and is red at physiological pH and above 0°C. Variation in color is normal, does not affect product efficacy, and will disappear upon equilibration with 5% CO_2.

4. **What is the normal appearance of thawed Corning Matrigel matrix?**

   A range of red-pink colors is expected for thawed products that contain phenol red. The vialed material should be clear (phenol red-containing and phenol red-free). Standard concentration products should be free-flowing (not gelled). High Concentration (HC) products are viscous.

5. **How long does it take to thaw Corning Matrigel matrix?**

   The product should be thawed overnight on ice in a 2°C to 6°C refrigerator (in the back), or cold room. It may take longer to thaw if the protein concentration is high.

6. **Do I really need to chill my pipet tips and tubes when using Corning Matrigel matrix?**

   Yes. Since Corning Matrigel matrix will start to form a gel above 10°C, we recommend the use of pre-cooled pipets, tips, and tubes when handling Corning Matrigel matrix.

7. **How quickly does Corning® Matrigel® matrix polymerize?**

   Corning Matrigel matrix will gel rapidly at 22°C to 35°C .

8. **Why would I use phenol red-free Corning Matrigel matrix?**

   Phenol red-free Corning Matrigel matrix is recommended for assays that require color detection. For example, it can be used with a fluorescent dye or Drabkins reagent to quantify endothelial cell tubulogenesis *in vivo*. For endometrial cultures, you must use a phenol red-free medium.

   Additionally, phenol red exhibits estrogenic effects. Phenol red bears a structural resemblance to some non-steroidal estrogens and has significant estrogenic activity. Moreover, it is a potential endocrine-disrupting compound that may have the capacity to interfere with the natural production and metabolism of hormones in the body of an experimental animal.

9. **How do I recover my cells from Corning Matrigel matrix? How do I choose between Corning Dispase and Corning Cell Recovery Solution?**

   Corning Dispase or Corning Cell Recovery Solution is recommended for recovering cells cultured on Corning Matrigel matrix.

   Corning Dispase will yield a single cell suspension more gently and effectively than trypsin, collagenase, or other proteolytic enzymes, as it will not damage cells or cleave cell surface proteins. Therefore, Dispase will not harm cells that are harvested for subcultivation or bioassays. In addition, Dispase may be used for tissue dissociation.

   Corning Cell Recovery Solution is recommended for metabolism experiments and RNA recovery. This reagent enables cell recovery using non-enzymatic procedure at 4°C. Since RNA is present in Matrigel matrix, a negative control sample (Matrigel matrix incubated in the absence of cells) should be included when performing RNA analysis.

   Other alternative possibilities to recover cells from Corning Matrigel matrix include:

   - Lowering the temperature between 4°C to 6°C to depolymerize the Matrigel matrix. This takes time and is not suitable for all applications.
   - Centrifugation to disrupt the Matrigel matrix.

10. **How long can I store a plate after it is coated with Corning Matrigel matrix?**

    It is always better to use Corning Matrigel matrix-coated plates the same day, but it is application dependent. Coated plates can be stored in the incubator at 37°C for up to a week in serum-free media.

11. **How much Corning Matrigel matrix do I use to coat a plate?**

Volume of Corning Matrigel matrix (µL/cm² growth area)

Thin Gel	Thick Gel
50	150 - 200

Cultureware	Growth area (cm²)*
6-well plate	9.6
24-well plate	2.0
96-well microplate	0.32
35 mm x 10 mm dish	11.78
100 mm x 20 mm dish	58.95

*Growth area for some of the most commonly used cultureware is listed in this table. A complete listing of culture vessel growth areas can be found at **www.corning.com/lifesciences**.

12. **What types of assays are recommended for Corning Matrigel matrix HC?**

    Corning Matrigel matrix, high concentration (HC) is suited for *in vivo* applications where a high protein concentration augments growth of tumors. The high protein concentration also allows the Matrigel matrix plug to maintain its integrity after subcutaneous injection into mice. This keeps the injected tumor cells and/or angiogenic compounds localized for in situ analysis and/or future excision.

13. How do you use Corning® Matrigel® matrix for 3D culture? How do you make a 3D gel? Do you need to embed the cells in Corning Matrigel matrix?

Corning Matrigel matrix, high concentration (HC) is suited for *in vivo* applications where one can use a thick gel for 3D cell culture on Matrigel matrix. Cells can be embedded within the Matrigel matrix or seeded on top of the Matrigel matrix layer (overlay method). Please refer to the *Corning Matrigel Matrix Guidelines for Use*, which can be found online for helpful information on the overlay method.

14. When can we use thin gel, thick gel, or a 3D culture method using Corning Matrigel matrix?

Thin gel is for cell attachment and proliferation. For applications such as propagation of primary cells that only need a protein layer and not a protein matrix, the thin layer (thin gel) method should be used.

Thick gel is used for 3D cell culture applications such as the differentiation of rat aorta tissue into capillary-like structures (Ring Assay), or cell invasion assays.

For applications where 3D like environment is desired to study cell-cell interactions or complex tissue-like structures, a 3D culture method should be used. Please refer to the *Corning Matrigel Matrix Guidelines for Use*.

15. What coating concentration should I use to study for endothelial tube formation?

For endothelial tube formation, the Corning Matrigel matrix concentration should be at least 10 mg/mL. For a 24-well plate coating, we recommend 0.289 mL of chilled Matrigel matrix (10 mg/mL) per well.

16. Do all types of Corning Matrigel matrix support hESC culture?

Not always. Corning offers hESC-qualified Corning Matrigel matrix (Corning Cat. No. 354277) which is QC tested for hESC maintenance to ensure consistency, reproducibility, and reliability in performance. This product has been qualified for use with STEMCELL Technologies' mTeSR®1 medium. Human embryonic stem cells were grown in mTeSR1 on Corning Matrigel matrix hESC-qualified matrix-coated plates for five passages and remained undifferentiated by standard morphology and surface marker expression.

In addition, Corning BioCoat™ Matrigel matrix 6-well plates (Corning Cat. No. 354671) are ready to use and Corning offers lot-to-lot consistency for culturing human ES cells while maintaining their ability for self-renewal and pluripotency.

While non-hESC-qualified Corning Matrigel matrix may work for this application, the results may vary when the product is not qualified for use with hES cells.

17. How much of Corning Matrigel matrix do I need for coating the cultureware for an invasion assay?

For coating a 24-well insert plate format, we recommend 0.1 mL (200 to 300 µg/mL) of Corning Matrigel matrix (Corning Cat. Nos. 354234, 354230) per insert.

18. What is the lowest concentration of Corning Matrigel matrix that will form a gel?

The optimal protein concentration is application dependent. Determine the protein concentration range that works best for your application. Corning Matrigel matrix diluted to a concentration of 3 mg/mL will form a gel. Do not dilute by fold dilution; dilute to a specific concentration (mg/mL).

To prevent incomplete gel formation for *in vivo* applications, do not dilute Corning Matrigel matrix to a final concentration below 4 mg/mL.

19. How long will a Corning® Matrigel® matrix plug last *in vivo*?

A Corning Matrigel matrix plug will last for at least one week *in vivo*.

Corning Matrigel Matrix Products and their Applications

Corning Matrigel Matrix	Type	Cat. No.	Size	Applications
Standard Corning Matrigel matrix	Standard	356234	5 mL	General cell culture[a]
		354234	10 mL	
		356235	50 mL (5 x 10 mL)	
		356232	25 mL (5 x 5 mL)	
		356254	100 mL (10 x 10 mL)	
	Phenol red-free[b]	356237	10 mL	General cell culture: Assays that require color detection (e.g., fluorescence).
	Growth Factor Reduced (GFR)[c]	356230	5 mL	General cell culture: The GFR product is useful for applications that benefit from a more highly defined basement preparation.
		354230	10 mL	
		356252	50 mL (5 x 10 mL)	
		356253	100 mL (10 x 10 mL)	
	GFR, Phenol red-free	356231	10 mL	General cell culture
		356238	50 mL (5 x 10 mL)	
		356239	100 mL (10 x 10 mL)	
HC Corning Matrigel matrix[d]	Standard	354248	10 mL	*In vivo* applications: Tumor formation, Corning Matrigel plug assay, angiogenesis; general cell culture
	Phenol red-free	354262	10 mL	
	GFR	354263	10 mL	
hESC-qualified Corning Matrigel matrix		354277	5 mL	hES culture, iPS culture
		356277	25 mL (5 x 5 mL)	
		356278	50 mL (10 x 5 mL)	

[a] General cell culture: Examples include 2D and 3D cultures, angiogenesis, and cell invasion assays. Standard concentration Corning Matrigel matrix products can also be used *in vivo* depending on the required protein concentration.
[b] Phenol red-free: Examples include *in vivo* angiogenesis assays when using fluorescent dye or Drabkins reagent to quantify vessel formation.
[c] GFR: Examples include signaling related studies, elucidating the role of growth factors, and gene expression studies.
[d] Corning Matrigel matrix HC: The HC formulation can also be used as an alternative to standard Corning Matrigel matrix when diluted to an appropriate concentration.

20. How do you dilute Corning Matrigel matrix?

Dilute Corning Matrigel matrix with ice-cold serum-free medium or phosphate-buffered saline (PBS), pH 7.4.

21. How do I pipet my Corning Matrigel matrix?

Use positive displacement pipets or syringes for accurate pipetting. Since Corning Matrigel matrix can stick to the inside and outside of warm pipets or syringes, the use of chilled pipets or syringes is strongly recommended.

Dispensing Tricks: Do not go to the bottom of the vial. Do not "blow out" the pipet or tip.

For Pipets: Dispense from 6 to 1 for 5 mL.

For Pipetman: Depress to the second stop to aspirate. Depress to the first stop to dispense.

22. Why is my Corning® Matrigel® matrix so viscous?

The higher the protein concentration the higher the viscosity. Concentrations over 13.0 mg/mL can be very thick. The Corning Matrigel matrix products will always exhibit extreme viscosity and will not become free-flowing until diluted. Matrigel matrix HC can be used undiluted with cells or for injection, or can be diluted to any protein range and used as any standard concentration Matrigel matrix product. The optimal protein concentration is application dependent.

Viscosity is also dependent on storage temperature. It is important to store Corning Matrigel matrix in a "non-frost-free" freezer. If stored IMPROPERLY in a "frost-free" freezer, the product will be exposed to freeze-thaw cycles and may become "clumpy." Freeze-thaws should be minimized by aliquoting into one time use aliquots. If product is frozen while gelled, it could be irreversibly gelled upon thawing. After thawing, the product should be held on ice.

23. Does Corning Matrigel matrix induce differentiation of ES/iPS cells?

Yes, Corning Matrigel matrix has been used to study differentiation of ES/iPS cells.

24. Why does Corning Matrigel matrix gel at 37°C, but becomes liquid at 4°C?

Corning Matrigel matrix is a reconstituted basement membrane extracted from EHS mouse tumor. When the material is extracted from the tumor, it contains laminin, collagen IV, entactin, heparan sulfate proteoglycan and growth factors that occur naturally in the EHS tumor. These proteins have multiple functional domains that interact with laminin, collagen IV, and heparin binding protein that contributes to the structural organization of the Matrigel matrix. Between 22°C to 37°C there is enough energy for the bonds to form and the Matrigel matrix gels. At 4°C there is not enough energy to form the bonds that would contribute to the structural organization of Matrigel matrix, so the Matrigel matrix liquefies or becomes a solution at this temperature.

25. Can Corning Matrigel matrix be used following multiple freeze-thaw cycles?

We recommend that freeze-thaw cycles be limited. Aliquots should be made when the vial is initially thawed and then stored appropriately.

26. My cells are not attaching; the gel is coming off the plate. What is wrong?

Check that the cell seeding density is not too high and that the amount of Matrigel matrix is equivalent to the volume of media used in the culture vessel. Matrigel matrix that is diluted to a very low concentration will form a weaker or more fragile gel and is more likely to detach from tissue culture plastic.

27. What can I do about precipitated matter in undiluted Corning Matrigel matrix?

Spin at a low speed at 4°C to pellet the precipitate prior to aliquoting the material.

28. How should I store remaining Corning Matrigel matrix that is not needed for the experiment?

We do not recommend storing remaining Corning Matrigel matrix once mixed with the media or buffer.

29. Does Corning Matrigel matrix contain DNA and/or RNA?

Yes. Corning Matrigel matrix is not DNase or RNase-treated. It contains trace amounts of DNA and RNA.

30. Does Corning Matrigel matrix contain VEGF and MMPs?

There is 5.0 to 7.5 ng/mL of VEGF in the standard Corning Matrigel matrix and 1.0 to 1.5 ng/mL of VEGF in GFR Corning Matrigel matrix. There are trace amounts of matrix metalloproteinase present in Corning Matrigel matrix that are derived from the mouse tumors cells.

31. Is Corning Matrigel matrix LDEV-free?

Yes. Corning Matrigel matrix has been tested and found negative for LDEV/LDHV using Mouse Antibody Production (MAP) and PCR analysis. In addition, we also screen mouse colonies and the tumor source for other viruses. The complete list is documented in the product specification sheet.

32. Is there any urea in Corning® Matrigel® matrix?

No. Urea is used in the preparation, but it is dialyzed out.

33. What buffer media is the Corning Matrigel matrix in?

Low-glucose DMEM (1g/L) containing 50 µg/mL gentamycin.

34. Does Corning Matrigel matrix contain fibronectin?

Yes. We have found trace amounts of fibronectin in Corning Matrigel matrix (detectable by Western blot).

35. Does Corning Matrigel matrix contain vitronectin?

Vitronectin could be present in trace amounts if blood was present in EHS tissue.

36. What else is in Corning Matrigel matrix?

Chloroform content (<0.02%) and undefined proteins/molecules derived from the tumor cells.

37. Does the extraction process cause the laminin to be denatured?

No. Laminin is in its native form; it is not denatured.

38. Can Corning Matrigel matrix be stored at -70°C?

Yes. Corning Matrigel matrix can be stored at -70°C but there are safety concerns regarding ultracold temperatures and glass vials. Matrigel matrix can be aliquoted and stored in polypropylene or other compatible tubes that can withstand -70°C.

39. What is the refractive index of Corning Matrigel matrix?

The refractive index of Corning Matrigel matrix is 1.3406 to 1.3407 at 20°C and that of water is 1.333 at 20°C, so the relative refractive index is 1.0056.

40. Does Corning Matrigel matrix have autofluorescence?

Corning Matrigel matrix is a protein solution dialyzed into DMEM and gentamycin. The protein component does fluoresce, but the excitation is in the UV range. DMEM does contain substances such as vitamins that may interfere with the experiment. We recommend that you perform a control experiment to determine background fluorescence.

41. How can I fix cells in Corning Matrigel matrix for applications requiring sectioning for subsequent immunohistochemical or immunofluorescence experiments? How can I avoid Corning Matrigel matrix de-polymerization after fixation?

Fix Corning Matrigel matrix with 2% paraformaldehyde. In some cases, Matrigel matrix tends to de-polymerize after fixation. Adding 1% glutaraldehyde to the Matrigel matrix can prevent depolymerization.

Glutaraldehyde is a fixative for electron microscopy and tends to generate significant background auto-fluorescence. We recommend adding a quenching step utilizing $NaBH_4$ after fixation (for immunofluorescence assays). Since $NaBH_4$ generates air bubbles in the process, this step should be performed on the bench without any shaking to minimize bubble formation. You can also try 0.1% to 0.5% glutaraldehyde to minimize or prevent depolymerization. The use of less glutaraldehyde may produce less background fluorescence.

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At Corning, cells are in our culture. In our continuous efforts to improve efficiencies and develop new tools and technologies for life science researchers, we have scientists working in Corning R&D labs across the globe, doing what you do every day. From seeding starter cultures to expanding cells for assays, our technical experts understand your challenges and your increased need for more reliable cells and cellular material.

It is this expertise, plus a 160-year history of Corning innovation and manufacturing excellence, that puts us in a unique position to offer a beginning-to-end portfolio of high-quality, reliable cell culture consumables.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

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