

Background

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), transforming growth factor (TGF-beta), epidermal growth factor (EGF), insulin-like growth factor (IGF-1), fibroblast growth factor (bFGF), 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 are the growth factor concentrations 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
IGF-1	11 - 24 ng/mL	15.6 ng/mL	5 ng/mL
TGF-b	1.7 - 4.7 ng/mL	2.3 ng/mL	1.7 ng/mL
EGF	0.5 - 1.3 ng/mL	0.7 ng/mL	<0.5 ng/mL
PDGF	5 - 48 pg/mL	12 pg/mL	<5 pg/mL
bFGF	<0.1 pg/mL	n.d.	n.d.
NGF	<0.2 ng/mL	n.d.	<0.2 ng/mL
VEGF	5.0 to 7.5 ng/mL	n.d.	1.0 to 1.5 ng/mL

n.d. = not determined

3. What type of DMEM is used in the manufacture of Corning Matrigel matrix?

Phenol red containing Matrigel: DMEM (1g/L glucose) containing 50 µg/mL gentamycin.

Phenol red-free Matrigel: DMEM (4.5g/L glucose) containing 50 µg/mL gentamycin.

4. 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 Certificate of Analysis.

5. Why is it important to use the lot-specific protein concentration of Corning Matrigel matrix?

Every lot of Corning Matrigel matrix has a specific protein concentration or dilution factor included on the Certificate of Analysis. The protein concentration varies between the lots and this specific protein concentration should be used to calculate the amount of Matrigel matrix required for your application.

6. 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.

7. Is there any urea in Corning Matrigel matrix?

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

8. Does Corning® Matrigel® matrix contain fibronectin?

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

9. Does Corning Matrigel matrix contain vitronectin?

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

10. What else is in Corning Matrigel matrix?

Chloroform content (<0.02%) and undefined proteins/molecules derived from the tumor cells. For more information on Corning matrigel composition see Matrigel: a complex protein mixture required for optimal growth of cell culture Proteomics 10(9):1886-90 2010

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

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

12. 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.

13. 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.

Thawing

14. 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% CO₂.



15. 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 vial 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 and not clear.

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

The product should be thawed overnight on ice in a 2°C to 8°C refrigerator (in the back), or cold room.

Handling

17. 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.

18. 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.

19. 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.

20. 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. Plates coated with Corning Matrigel hESC-qualified matrix should be stored at 4°C for up to a week in Matrigel coating solution.

21. 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.

22. 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.

23. 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.

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.

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. 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.

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

Corning Matrigel matrix that is diluted to a very low concentration (e.g., less than 3 mg/mL) will form a weaker or more fragile gel and is more likely to detach from tissue culture plastic. Optimize cell seeding density as high seeding densities may impair gelation.

27. 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 in a -20°C or -70°C freezer.

28. Can I store undiluted Corning Matrigel matrix at 4°C after thawing/dilution?

We do not recommend long-term storage of Corning Matrigel matrix at 4°C post-thaw/-dilution.

29. 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.

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

Recommended Volume of Corning Matrigel Matrix (µL/cm² growth area)

Thin Gel	Thick Gel
50	150 - 200

Cultureware	Growth area (cm ²)*
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.

31. 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 a non-enzymatic procedure at 4°C. Since RNA is present in Corning Matrigel matrix, a negative control sample (Corning 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 8°C to depolymerize the Matrigel matrix. This takes time and is not suitable for all applications.
- ▶ Centrifugation to disrupt the Matrigel matrix.

Applications

32. What coating concentration should I use to study 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.

33. How much Corning Matrigel matrix do I need for coating 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 (Cat. No. 354234 or 354230) per insert.

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

Not always. Corning offers hESC-qualified Corning Matrigel matrix (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 medium on Corning Matrigel hESC-qualified matrix-coated plates for five passages and remained undifferentiated by standard morphology and surface marker expression.

In addition, Corning BioCoat™ Matrigel 6-well plates (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.

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

It depends on the concentration and the media used, Corning Matrigel matrix at the proper concentrations with the proper media can maintain the proliferative phenotype and prevent differentiation of stem cells. Corning Matrigel matrix can also be used to induce differentiation.

36. How do you use Corning Matrigel matrix for 3D culture?

3D culture can be achieved using a thick gel of Matrigel matrix to culture cells on top (overlay method) or embedded within the gel. Other thick gel Matrigel matrix culture methods include 3D dome or sandwich techniques commonly used for organoid culture.

37. What is the difference between embedded method vs. sandwich method vs. dome formation methods?

Embedded culture involves cells being mixed directly with Corning Matrigel matrix for organoid culture prior to dispensing, it results in a single thick layer of Matrigel matrix with cells.

The sandwich method requires a layer of Matrigel matrix to be dispensed into the cultureware and polymerized prior to adding cells in media containing a dilute concentration of Matrigel matrix. This method results in cells that sit on top of the Matrigel matrix and are in a narrower focal plane, making this method ideal for imaging applications.

Dome formation method involves formation of droplets of cell suspension containing Matrigel matrix for organoid culture on a TC-treated surface. This method is typically used for organoid culture protocols.

Please refer to the respective *Corning Matrigel Matrix for Organoid Culture Guidelines for Use (Corning Lit. Code SPC-356255-G)*, which can be found online (www.corning.com/lifesciences) for helpful information on each method.

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

Thin gel is typically used for cell attachment and proliferation applications. 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 such as organoid culture and differentiation studies, a 3D culture method should be used.

Please refer to the *Corning Matrigel Matrix for Organoid Culture Guidelines for Use (Corning Lit. Code SPC-356255-G)*.

39. Do all types of Corning Matrigel matrix support organoid culture?

Not always; While other Corning Matrigel matrix products can work for this application, the results may vary. Corning Matrigel matrix for organoid culture provides a consistent and reliable platform for the culture of organoids. It has been verified to support mouse intestinal organoid growth for more than 7 passages with typical budding morphology and marker expression (see Application Note, Corning Lit. Code CLS-AN-542). It has also been verified to support polarized 3D structures of primary human airway epithelial cells expressing typical markers (see Application Note, Corning Lit. Code CLS-AN-534). Additionally, each lot is measured for its elastic modulus indicative of gel stiffness and is qualified to form stable "3D domes" commonly used in organoid culture protocols.

40. Can you describe the dome formation and maintenance assay mentioned on the Corning® Matrigel® matrix for organoid culture Certificate of Analysis?

Each lot of Corning Matrigel matrix for organoid culture is tested for its ability to form and maintain droplets (domes) on a pre-incubated TC-treated 24-well surface (Cat. No. 3524 or 3526). The 50 µL droplets are formed with Corning Matrigel matrix for organoid culture (diluted to 7 mg/mL with DMEM), allowed to polymerize and subsequently 750 µL of DMEM is added to each well. These droplets are maintained for a period of 7 days in a humidified incubator at 37°C.

41. Do plate type, manufacturer, surface, and conditioning influence the formation and maintenance of the domes during organoid culture?

Yes, for organoid protocols that require dome formation, factors such as plate type and plate pre-incubation are important parameters. We recommend using TC-treated plates (Corning 24-well plates, Cat. No. 3524 or 3526) and pre-incubation in a humidified incubator overnight for dome formation. Use inside wells for best success.

42. What can I do to minimize breaking the domes while I transport the plate to the incubator?

We recommend placing the plate on a heated platform (Corning LSE™ Digital dry bath heater, dual block [Cat. No. 6885-DB] with a Corning LSE Dual block only, 96-well microplate, or 4 slides [Cat. No. 480131]) during dome formation. The heated platform would ensure that the plate is uniformly heated and would allow the Corning Matrigel matrix droplet to polymerize before transporting the plate to the incubator. Avoid any sudden movements during plate transportation and placement in the incubator.

43. Can I add cold medium to the well containing the Corning Matrigel matrix dome?

Please use room temperature medium after the Matrigel matrix for organoid culture dome droplet has polymerized to avoid disrupting the Corning Matrigel matrix dome.

44. Do any reagents impact Corning Matrigel matrix polymerization during dome formation and maintenance?

Certain reagents used for cell dissociation can disrupt the polymerization of Matrigel matrix for organoid culture. It is imperative to adequately wash the cells/clumps/fragments before adding Matrigel matrix for organoid culture to cell/organoid suspension.

45. What is elastic modulus?

Elastic modulus is a measure of the response of a material when a mechanical force (e.g., shear force or tensile force) is applied to it¹. For example, a stiffer material has a higher elastic modulus and changes its shape only slightly, whereas a flexible material has a lower elastic modulus.

46. Why is elastic modulus (material stiffness) important for organoid culture?

Researchers have reported that mechanical rigidity of a matrix, to which cells adhere, plays a significant role in organoid formation and expansion, because cells are able to sense and respond to changes in their mechanical environment². For instance, stiffness of cell adherent matrix can modulate cardiomyogenic and endothelial differentiation of embryoid bodies, and further tailor the internal structure of cardiovascular organoids³. Soft hydrogels enhance the formation of kidney organoids⁴; mechanical as well as biochemical properties of the matrix are important for epithelial organoid expansion^{5,6}.

47. How do you measure the elastic modulus of Corning Matrigel matrix for organoid culture?

Elastic modulus values of Matrigel matrix are dependent on the type of instrument used and the method used for measurement. Elastic modulus for each lot of Corning Matrigel matrix for organoid culture is measured by single frequency oscillatory measurements using a parallel plate rotational rheometer using a proprietary method. The plateau of the elastic component of the shear modulus (G') is reported as the elastic modulus in Pascals. The elastic modulus is measured for gelled undiluted Matrigel matrix for organoid culture.

48. How does the elastic modulus of Corning Matrigel matrix for organoid culture compare to that for other Corning Matrigel matrix products?

Currently, elastic modulus is only reported for Corning Matrigel matrix for organoid culture. Data shows of the effect of protein concentration on the elastic moduli of Corning high concentration Matrigel matrix and Collagen I gels using a rotational rheometer (Corning Lit. Code CLS-AC-AN-449).

49. What types of applications require 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.

50. 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*.

51. 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 depolymerization after fixation?

We recommend fixing cells growing in Corning Matrigel matrix with 2% paraformaldehyde. In some cases, Matrigel matrix tends to depolymerize 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 NaBH₄ after fixation (for immunofluorescence assays). Since NaBH₄ generates air bubbles in the process, this step should be performed on the bench without any shaking to minimize bubble formation. You could also try a lower concentration of glutaraldehyde (0.1% to 0.5%) to minimize depolymerization. The use of less glutaraldehyde may produce less background fluorescence.

Corning Matrigel Matrix Products and their Applications

Corning Matrigel Matrix	Type	Corning Cat. No.	Size	Applications	
Standard Corning Matrigel Matrix	Phenol red	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).	
		356230	5 mL		
	Growth Factor Reduced (GFR) ^c	GFR	354230	10 mL	General cell culture: The GFR product is useful for applications that benefit from a more highly defined basement preparation.
			356252	50 mL (5 x 10 mL)	
			356253	100 mL (10 x 10 mL)	
GFR, Phenol red-free	GFR	356231	10 mL	General cell culture	
		356238	50 mL (5 x 10 mL)		
		356239	100 mL (10 x 10 mL)		
High Concentration Corning Matrigel Matrix ^d	Phenol red	354248	10 mL	<i>In vivo</i> applications: Tumor formation, Corning Matrigel matrix plug assay, angiogenesis; general cell culture	
	Phenol red-free	354262	10 mL		
	GFR, phenol red	354263	10 mL		
hESC-qualified Corning Matrigel Matrix	Phenol-red	354277	5 mL	hESC culture, hiPSC culture	
		356277	25 mL (5 x 5 mL)		
		356278	50 mL (10 x 5 mL)		
Corning Matrigel Matrix for Organoid Culture	Phenol red-free	356255	10 mL	Organoid culture and differentiation	

^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.

References

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

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