

BD Matrigel matrix

Frequently Asked Questions.

What is BD Matrigel matrix?

BD 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. BD 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 is also residual matrix metalloproteinases derived from the tumor cells.

What is the growth factor (GF) concentration in BD Matrigel matrix?

Amounts of Growth Factors (GF) Present in BD Matrigel matrix vs. Growth Factor Reduced (GFR) BD Matrigel matrix			
Growth Factor	Range of GF Concentration in BD Matrigel matrix	Average GF Concentration in BD Matrigel matrix	Typical GF Concentration in GFR BD 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			

Why is my BD Matrigel matrix different colors?

Color variations may occur in frozen or thawed vials of BD 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.

What is the normal appearance of thawed BD 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.

How long does it take to thaw BD 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.

Do I really need to chill my pipet tips and tubes when using BD Matrigel matrix?

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

How quickly does BD Matrigel matrix polymerize?

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

Why would I use phenol red-free BD Matrigel matrix?

Phenol red-free BD Matrigel matrix is recommended for assays that require color detection. For example, when using 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.

How do I recover my cells from BD Matrigel matrix? How do I choose between BD Dispase and BD Cell Recovery Solution?

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

BD 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, BD Dispase will not harm cells that are harvested for subcultivation or bioassays. In addition, BD Dispase may be used for tissue dissociation.

BD 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 BD Matrigel matrix, a negative control sample (BD Matrigel incubated in the absence of cells) should be included when performing RNA analysis.

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

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

How long can I store a plate after it is coated with BD Matrigel matrix?

It is always better to use BD 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.

How much BD Matrigel matrix do I use to coat a plate?

Volume of BD Matrigel™ matrix (µl/cm ² 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 plate	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 on the BD Biosciences online catalog.

What types of assays are recommended for BD Matrigel matrix HC?

BD 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 BD 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.

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

One can use a thick gel for 3D cell culture on BD Matrigel matrix. Cells can be embedded within the BD Matrigel matrix or seeded on top of the BD Matrigel matrix layer (overlay method). Please refer to the BD Matrigel matrix Guidelines for Use, and Application Note 476 which both can be found online for helpful information on the overlay method.

When can we use thin gel, thick gel, or a 3D culture method using BD 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 BD Biosciences Guidelines for BD Matrigel matrix gel.

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

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

Do all types of BD Matrigel matrix support hESC culture?

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

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

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

How much of BD 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 (200-300 µg/ml) of BD Matrigel matrix (BD cat. nos. 354234, 354230) per insert.

What is the lowest concentration of BD 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. BD 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 BD Matrigel matrix to a final concentration below 4 mg/ml.

How long will a BD Matrigel matrix plug last in vivo?

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

BD Matrigel matrix Products and their Applications

BD Matrigel matrix	Type	BD cat. no.	Applications
Standard BD Matrigel matrix	Standard	356234 354234 356235	General cell culture*.
	Phenol red-free■	356237	General cell culture; assays that require color detection (e.g., fluorescence).
	Growth Factor Reduced (GFR)▲	356230 354230	General cell culture. The GFR product is useful for applications that benefit from a more highly defined basement preparation.
	GFR, Phenol red-free	356231	General cell culture.
HC BD Matrigel matrix✦	Standard	354248	In vivo applications: tumor formation, BD Matrigel plug assay, angiogenesis; general cell culture.
	Phenol red-free	354262	
	GFR	354263	
hESC-qualified BD Matrigel matrix		354277	hES culture; iPS culture.

***General cell culture:** examples include 2D and 3D cultures, angiogenesis, and cell invasion assays. Standard concentration BD Matrigel™ matrix products can also be used in vivo depending on the required protein concentration.

▪**Phenol red-free:** examples include in vivo angiogenesis assays when using fluorescent dye or Drabkins reagent to quantify vessel formation.

▲**GFR:** examples include signaling related studies, elucidating the role of growth factors, and gene expression studies.

✦**BD Matrigel matrix HC:** The HC formulation can also be used as an alternative to standard BD Matrigel matrix diluting to appropriate concentration.

How do you dilute BD Matrigel matrix?

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

How do I pipet my BD Matrigel matrix?

Use positive displacement pipet or syringe for accurate pipeting. Since BD 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 vial. Do not “blow out” the pipet or tip.

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

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

Why is my BD Matrigel matrix so viscous?

The higher the protein concentration the higher the viscosity. Concentrations over 13.0 mg/ml can be very thick. The BD Matrigel matrix products will always exhibit extreme viscosity and will not become free-flowing until diluted. BD 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 BD Matrigel matrix product. The optimal protein concentration is application dependent.

Viscosity is also dependent on storage temperature. It is important to store BD 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.

Does BD Matrigel matrix induce differentiation of ES/iPS cells?

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

Why does BD Matrigel matrix gel at 37°C, but becomes liquid at 4°C?

BD 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 BD Matrigel matrix. Between 22°C to 37°C there is enough energy for the bonds to form and the BD Matrigel matrix gels. At 4°C there is not enough energy to form the bonds that would contribute to the structural organization of BD Matrigel matrix, so the BD Matrigel matrix liquifies or becomes a solution at this temperature.

Can BD 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.

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 BD Matrigel™ matrix is equivalent to the volume of media used in the culture vessel. BD 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.

What can I do about precipitated matter in undiluted BD Matrigel matrix?

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

How should I store remaining BD Matrigel matrix that is not needed for the experiment?

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

Does BD Matrigel matrix contain DNA and/or RNA?

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

Does BD Matrigel matrix contain VEGF and MMPs?

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

Is BD Matrigel matrix LDEV-free?

Yes. BD 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.

Is there any urea in BD Matrigel matrix?

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

What buffer media is the BD Matrigel matrix in?

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

Does BD Matrigel matrix contain fibronectin?

Yes, we have found trace amounts of fibronectin in BD Matrigel matrix (detectable by Western blot).

Does BD Matrigel matrix contain vitronectin?

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

What else is in BD Matrigel matrix?

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

Does the extraction process cause the laminin to be denatured?

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

Can BD Matrigel matrix be stored at -70°C?

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

What is the refractive index of BD Matrigel matrix?

The refractive index of BD 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.

Does BD Matrigel matrix have autofluorescence?

BD 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.

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

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

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 can also try 0.1% to 0.5% glutaraldehyde to minimize or prevent depolymerization. The use of less glutaraldehyde may produce less background fluorescence.

