

CERTIFICATE OF ANALYSIS

PRODUCT: Corning® Matrigel® Matrix for Organoid Culture, Phenol Red-free, 10 ml Vial
CATALOG NUMBER: 356255

LOT NUMBER: 34424001

SOURCE: Engelbreth-Holm-Swarm (EHS) Mouse Tumor
FORMULATION: Dulbecco's Modified Eagle's Medium with 50 µg/mL gentamycin.

ORGANOID CULTURE: Corning Matrigel matrix for organoid culture has been verified to support the growth of mouse intestinal organoids for more than 7 passages with typical budding morphology and marker expression¹ using HUB Organoid Technology methods*. This product has also been verified to support the formation of polarized 3D structures of primary human airway epithelial cells expressing typical markers².

STORAGE: Store at -20°C. Avoid multiple freeze-thaws. Do not store in frost-free freezer. **KEEP FROZEN.**

QUALITY CONTROL:

Specification	Criteria	Result
Protein Concentration	Results obtained by Lowry method and represented in mg/mL.	9.2
Endotoxin	Endotoxin units (EU/mL) are measured by Limulus Amoebocyte Lysate assay.	<1.5
Elastic Modulus (matrix stiffness)	Elastic modulus (Pa) is measured by single frequency oscillatory measurements using a parallel plate rotational rheometer. The plateau of the elastic component of the shear modulus (G') at 37°C is reported as the elastic modulus.	81.2
Dome Formation and Maintenance	Tested for ability to form and maintain droplets on pre-incubated TC-treated 24-well surface. The 50 µL droplets were made with Corning Matrigel matrix for organoid culture (diluted to 7 mg/mL with DMEM) and 750 µL of DMEM was added to each well. These droplets were maintained for a period of 7 days in a humidified incubator at 37°C.	PASS
Gelling	Tested for ability to gel quickly and maintain this form with culture medium for a period of 14 days at 37°C.	PASS
Biological Activity	Biological activity is determined using a neurite outgrowth assay. Chick dorsal root ganglia are plated on a 1.0 mm layer of Corning Matrigel matrix. Tested for a positive neurite outgrowth response after 48 hours without addition of nerve growth factor.	PASS
Sterility	Tested for the presence of bacteria, fungi, and mycoplasma.	NEGATIVE
LDEV/LDHV	Finished goods tested for the presence of Lactate Dehydrogenase-Elevating Virus	NEGATIVE

Mouse colonies and/or tumor source are tested via serology or PCR and found to be negative for the following: LDEV/LDHV, Sendai Virus, Mouse Hepatitis Virus (MHV), Pneumonia Virus of Mice (PVM), Theiler's Virus (TMEV/GDVII), Ectromelia Virus, Polyoma Virus, Epizootic Diarrhea of Infant Mice (EDIM), Lymphocytic Choriomeningitis Virus (LCM), Murine Cytomegalovirus (MCMV), Mouse Adenovirus (1,2), Respiratory Enteric Virus (1, 2, 3), Mouse Parvovirus (MPV), Mouse Thymic Virus (MTV), Hantaan Virus, K Virus, Cilia Associated Respiratory Bacillus (CARB), Mycoplasma pulmonis, Helicobacter Spp., Mouse Minute Virus (MMV), Murine Norovirus.

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SAFETY RECOMMENDATION: Handle in accordance with good industrial hygiene and laboratory safety practices

Expiration Date: February 16, 2027

Quality Assurance:

Eugene B. Hartigan

Date:

January 28, 2025

References:

1. Application note (Corning Lit. Code CLS-AN-542). Culture of mouse intestinal organoids in Corning Matrigel matrix for organoid culture.
2. Application note (Corning Lit. Code CLS-AN-534). High throughput gene expression analysis of 3D airway organoids.

*Should you intend to use the HUB organoid technology for commercial purposes, please contact the HUB at info@hub4organoids.nl.

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GUIDELINES FOR USE

PRODUCT: Corning® Matrigel® Matrix for Organoid Culture, Phenol Red-free, 10 mL Vial

CATALOG NUMBER: 356255

BACKGROUND: Basement membranes are continuous sheets of specialized extracellular matrix (ECM) that are found at the dermal-epidermal junction, at the base of all lumen-lining epithelia.

Corning Matrigel matrix for organoid culture is a soluble basement membrane extract of the Engelbreth-Holm-Swarm (EHS) tumor that gels at room temperature to form a genuine reconstituted basement membrane¹. The major components of Corning Matrigel matrix are laminin, collagen IV, entactin, and heparan sulfate proteoglycan, growth factors, collagenases, plasminogen activators, and other undefined components²⁻⁴.

ORGANOIDS: “An organoid is a collection of organ-specific cell types that develops from progenitors or stem cells, which self-organize through cell sorting and lineage commitment mimicking mini-organs”⁵. They can be classified into those that are tissue-derived and those derived from pluripotent stem cells (PSC). ECM is an important component of the cell niche that provides biochemical cues and structural support (porosity and stiffness) which mediates signaling for cell migration, cell behavior, and polarization in organoid structures^{6,7}. “When stem cells are placed within Corning Matrigel matrix and in the presence of suitable exogenous factors, they can be coaxed to form organized 3D clusters of cells”⁸.

Corning Matrigel matrix for organoid culture is an optimal matrix that has been verified to support organoid growth and differentiation. Each lot is measured for its elastic modulus indicative of matrix stiffness and qualified to form stable “3D dome” structures commonly used in organoid culture protocols. The product has been verified to support mouse intestinal organoid culture for more than 7 passages with typical budding morphology and marker expression⁹, it has also been verified to support the formation of polarized 3D structures of primary human airway epithelial cells expressing typical markers¹⁰.

Corning Matrigel matrix for organoid culture will provide customers the convenience of an optimized matrix, reducing the need for time-consuming screening, while providing the reproducibility and consistency essential for organoid research.

SOURCE: Engelbreth-Holm-Swarm (EHS) Mouse Tumor

FORMULATION: Dulbecco's Modified Eagle's Medium with 50 µg/mL gentamycin.

STORAGE: Stable when stored at -20°C. Store aliquots in either the -20°C or -70°C freezer until ready for use. Freeze thaws should be minimized by aliquoting into one time use aliquots. **Do not store in frost-free freezer. Keep frozen.**

PROTEIN CONCENTRATION: The protein concentration for Corning Matrigel matrix for organoid culture is lot-specific and can be found on the product Certificate of Analysis.

EXPIRATION DATE: The expiration date for Corning Matrigel matrix for organoid culture is lot-specific and can be found on the product Certificate of Analysis.

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THAWING:

CAUTION: Corning® Matrigel® matrix for organoid culture should always be kept submerged in ice, as it will start to gel above 10°C.

- Thaw vial by submerging it in an insulated bucket filled with ice.
- Keep the ice bucket in the back of a 2°C to 8°C refrigerator overnight.
- Once thawed, swirl the vial to ensure that material is evenly dispersed and spray the top of the vial with 70% ethanol while keeping material on ice.
- Using sterile technique, dispense the material into appropriate aliquots in pre-chilled tubes to avoid freeze-thaw cycles, and refreeze immediately.

METHODS OF USE:

Protocols for organoid culture should be optimized based on cell type and culture conditions. Typically, organoids are grown in Corning Matrigel matrix domes and in some instances, using a thick gel of Corning Matrigel matrix.

Dome application method

- We recommend Corning TC-treated cultureware (e.g., Cat. No. 3524 or 3526 for 24-well plates).
- We recommend preincubating the cultureware in a humidified 37°C incubator overnight.
- Adequately rinse the cell/organoid pellet to remove residual dissociating reagent before mixing with ice-cold Corning Matrigel matrix for organoid culture.
 - Residual dissociation reagents can affect dome formation.
 - We recommend that the cell/matrix mixture contain at least 50% Corning Matrigel matrix for organoid culture. Depending on organoid types and protocols, this mixture may require a higher concentration of Corning Matrigel matrix (>50%).
 - Avoid introduction of air bubbles while mixing.
- Domes are typically made by carefully dispensing 5 to 50 µL of the above-mentioned mixture.
- During dome formation, we recommend placing the multiwell plate on a heated platform (e.g., Corning Dry Bath, Cat. No. 6885-DB; with Heat Block, Cat. No. 480131) to maintain temperature.
- We recommend optimizing dome polymerization time based on volume, final Corning Matrigel matrix protein concentration, and culture medium. Typically, domes take at least 10 min. to solidify.
- We recommend gently adding room temperature organoid culture medium after dome solidification along the well wall, to avoid disrupting the domes.
 - If all wells are not used, add sterile PBS to empty wells to avoid dehydration.
- Carefully aspirate the medium to avoid disruption of the domes during medium changes and passaging.

Thick gel application method

- Keep culture plates and pipet tips on ice.
- Thaw Corning Matrigel matrix for organoid culture as recommended above.
- Add Corning Matrigel matrix for organoid culture (150 to 200 µL/cm²) to the plate.
- Allow the matrix to polymerize in a humidified incubator at 37°C for at least 30 min.
- Add cell suspension in culture medium on top of the gel.
 - Addition of Corning Matrigel matrix in medium may be needed for some organoid applications. This is referred to as a sandwich method.

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Embedded application method

- Keep culture plates and pipet tips on ice.
- Thaw Corning® Matrigel® matrix for organoid culture as recommended above.
- Resuspend and mix cell pellet with ice-cold Corning Matrigel matrix for organoid culture.
- Dispense mixture onto cold culture plate (150 to 200 $\mu\text{L}/\text{cm}^2$).
- Allow the matrix to polymerize in a humidified incubator at 37°C for at least 30 min. Add culture medium on top of the gel

NOTE: For more details on specific applications of Corning Matrigel matrix, visit our resources page at <https://www.corning.com/worldwide/en/products/life-sciences.html> for application notes/technical bulletins, protocols, and frequently asked questions.

SAFETY RECOMMENDATION: Handle in accordance with good industrial hygiene and laboratory safety practices.

REFERENCES:

1. Kleinman HK, et al. Basement membrane complexes with biological activity. *Biochemistry*, 25:312 (1986).
2. Kleinman HK, et al. Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma. *Biochemistry*, 21:6188 (1982).
3. Bissell DM, et al. Support of cultured hepatocytes by a laminin-rich gel. Evidence for a functionally significant subendothelial matrix in normal rat liver. *J Clin Invest*, 79(3):801 (1987).
4. Vukicevic S, et al. Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components. *Exp Cell Res*, 202:1 (1992).
5. Lancaster MA and Knoblich JA. Organogenesis in a dish: Modeling development and disease using organoid technologies. *Science*, 345:1247125 (2014).
6. Hartman CD, et al. Extracellular matrix type modulates cell migration on mechanical gradients. *Experimental Cell Research*, vol. 359(2):361-366 (2017).
7. Bryant DM, et al. A molecular switch for the orientation of epithelial cell polarization. *Developmental Cell*, 31(2):171-187 (2014).
8. Yin X, et al. Engineering stem cell organoids. *Cell Stem Cell* 18(1):25-38 (2016).
9. Application note (Corning Lit. Code CLS-AN-542), Culture of mouse intestinal organoids in Corning Matrigel matrix for organoid culture.
10. Application note (Corning Lit. Code CLS-AN-534), High throughput gene expression analysis of 3D airway organoids.



California Proposition 65 Notice

WARNING

This product contains a chemical known to the state of California to cause cancer. Component: Chloroform.

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