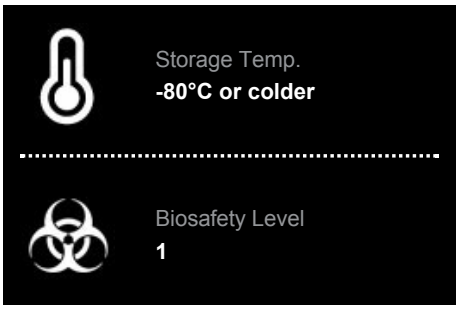
ATCC

Product Sheet

CellMatrix Basement Membrane Gel (ATCC® ACS-3035™)

Please read this FIRST



Description

Product Description:
Volume: 5 mL
Storage: -80°C
CellMatrix Basement Membrane Gel is a soluble, growth factor reduced basement membrane extract that does not contain phenol red. CellMatrix Gel is purified from murine Engelbreth-Holm-Swarm (EHS) tumor. Major components include laminin, collagen IV, entactin and heparin sulfate proteoglycan.

Volume: 5 mL

Directions for Use

Preparation and storage
Critical: CellMatrix Membrane Gel must remain frozen at all times during storage. Once thawed, keep CellMatrix on ice at all times. Do not allow it to warm above 4°C or premature gelation will occur.
Procedure

1. Thaw stock CellMatrix Membrane Gel vial on ice in a 4°C refrigerator overnight.
2. Determine the appropriate volume per aliquot based on concentration and application. Please refer to the certificate of analysis for the lotspecific concentration.
3. Dispense appropriatelysized aliquots using precooled tips into precooled tubes on ice and re-freeze immediately.
4. Thaw aliquots on ice. Once thawed, do not re-freeze. Once thawed, use within 7 days.

A. Three-dimensional culture of organoids in CellMatrix

1. Thaw an aliquot of CellMatrix on ice and keep on ice.
2. Resuspend dissociated organoids (fragments or single cells) in undiluted CellMatrix. Do introduce bubbles when pipetting. Work quickly to ensure the suspension remains cold.
3. Dispense as small droplets in a pre-warmed (in a 37°C cell culture incubator) tissue culture treated vessel (e.g., 50 µL per well in a 24-well plate). Work quickly to prevent premature gelation.
4. Place the plate in a 37°C cell culture incubator for 15-30 minutes to induce gelation.
5. Add an appropriate volume of organoid culture media.

Harvesting organoids from CellMatrix

1. Aspirate the media from the well completely
2. Using a cell lifter scrape the well surface to release the gelled domes
3. Transfer the domes to a 15 conical tube.
4. Pipet up and down 20-30 times to break up the domes and release the organoids from the CellMatrix gel.
5. Wash the organoids in cold DMEM:F12 (ATCC 30-2006) by centrifugation.
6. Aspirate the supernatant leaving behind the pellet of organoids.
7. Proceed with downstream assays using the organoids or resuspend in fresh CellMatrix to continue culture.

B. Sandwich culture of human hepatocytes

1. Thaw hepatocytes and seed on collagen type-1 coated plates.
2. After 6-24 hours, perform a complete media change with cold hepatocyte maintenance media that has been supplemented with 0.25 mg/mL CellMatrix. Refer to the certificate of analysis for the lotspecific concentration. For example, if the lot-specific concentration of CellMatrix is 15 mg/mL, add 16 uL CellMatrix per mL of hepatocyte culture media.
3. Return plate to the incubator.
4. After 24 hours perform a complete media change with hepatocyte maintenance media (not supplemented with CellMatrix).

C. Angiogenesis modeling via the tube formation assay

1. Thaw CellMatrix Gel in the refrigerator (2°C to 8°C), in ice, overnight.
2. Mix well by carefully pipetting CellMatrix Gel up and down, being careful not to introduce air bubbles.
3. Do not dilute. Pipette 150 µL/cm2 onto the tissue culture vessel surface.
4. Swirl the vessel gently to ensure that the entire surface is evenly covered.
5. Leave the coated dishes at 37°C for 30 minutes.
6. The coated vessels are ready for use.

D. Feeder-free culture of human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC)
Refer to the ATCC Stem Cell Culture Guide for more information.
Important: CellMatrix Gel will solidify in 15 to 30 minutes if the temperature is above 15°C. Keep CellMatrix Gel and labware (pipette tips, serological pipettes, conical tubes) on ice at all times to prevent the matrix from gelling prematurely. If air bubbles form when coating the dishes, use a chilled pipette tip to break up the bubbles.
For stem cells, NPCs, NPCs-derived neurons:

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

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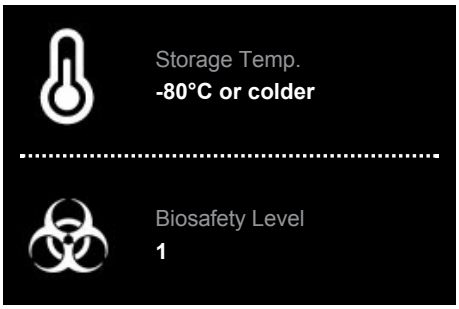

1. Thaw CellMatrix Gel in the refrigerator (2°C to 8°C), in ice, overnight.
2. Dilute the thawed CellMatrix Gel to 150 µg/mL by directly adding CellMatrix in cold DMEM: F12 Medium (ATCC 30-2006) on ice and mix well. Immediately coat each 6 cm dish with 2 mL diluted CellMatrix Gel.
3. Swirl dish gently to ensure that the entire dish is evenly covered.
4. Leave the coated dishes at 37°C for one hour.
5. Aspirate the coating solution and immediately plate the cells. It is critical that the coating does not dry out.
6. If the dishes will not be used the same day they are prepared, do not aspirate the coating solution. Seal the coated dishes with parafilm and store at 2°C to 8°C for up to one week. Note that stored dishes should be warmed to room temperature in a biological safety cabinet for at least one hour before use.

References

1. ATCC Angiogenesis Technical Bulletin.
2. Albini A, et al. A rapid in vitro assay for quantitating the invasive potential of tumor cells. Cancer Res 47(12): 3239-3245, 1987.
3. Kubota Y, et al. Role of laminin and basement membrane in the morphological differentiation of human endothelial cells into capillary-like structures. J Cell Biol 107:1589-1598, 1988.
4. Ponce M, et al. Identification of endothelial cell binding sites on the laminin gamma 1 chain. Circ Res 84:688-694, 1999.
5. Eisenstein M, Thinking outside the dish. Nat Methods 3:1035-1043, 2006.
6. Arnaoutova I, et al. The endothelial cell tube formation assay on basement membrane turns 20: state of the science and the art. Angiogenesis 12(3):267-274, 2009.
7. Angel M, Yanik MF. Innate immune suppression enables frequent transfection with RNA encoding reprogramming proteins. PLoS ONE. 5(7):e11756, 2010.

Quality Control Specifications

Functional testing: Each lot of CellMatrix Gel is shown to promote the attachment of hESCs/hiPSCs and to maintain hESCs/hiPSCs in a pluripotent state as determined by the expression of Oct-4 and Nanog pluripotent markers.

Sterility testing: No bacterial or fungal growth is detected. Negative for the presence of mycoplasma and 31 pathogens and viruses, including LDEV, as demonstrated by PCR. Endotoxin concentrations are less than 8 EU/mL by LAL assay.

Concentration: 12 to 18 mg/mL. Please refer to the certificate of analysis for the lot-specific concentration.

Storage Buffer: Product is stored in Dulbecco's Modified Eagle's Medium (DMEM) with 10 µg/mL gentamycin, without phenol red. A Certificate of Analysis is available upon request.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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