Cell Basement Membrane

ACS-3035™

Description

Cell Basement Membrane is purified from murine Engelbreth-Holm-Swarm (EHS) tumor. Cell Basement Membrane has applications in the three-dimensional culture of primary tissue-derived human and mouse organoids, a 3-D culture of iPSC-derived organoids, patient-derived organoids, a 3-D culture of tumorspheres from various cell lines, and a sandwich culture of human hepatocytes. Cell Basement Membrane is a feeder-free culture of human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC), a culture of iPSCs-derived neural progenitor cells (NPCs) and neurons, and angiogenesis modeling via the tube formation assay.

Volume: 5.0 mL

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Handling Procedures

Preparation and storage

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

Procedure

1. Thaw stock Cell Basement Membrane 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 Cell Basement Membrane

1. Thaw an aliquot of Cell Basement Membrane on ice and keep on ice.

2. Resuspend dissociated organoids (fragments or single cells) in undiluted Cell Basement Membrane. 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 Cell Basement Membrane

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 Cell Basement Membrane.
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 Cell Basement Membrane 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 Cell Basement Membrane. Refer to the certificate of analysis for the lot specific concentration. For example, if the lot-specific concentration of Cell Basement Membrane is 15 mg/mL, add 16 uL Cell Basement Membrane 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 Cell Basement Membrane).

C. Angiogenesis modeling via the tube formation assay

1. Thaw Cell Basement Membrane in the refrigerator (2°C to 8°C), in ice, overnight.
2. Mix well by carefully pipetting Cell Basement Membrane 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: Cell Basement Membrane will solidify in 15 to 30 minutes if the temperature is above 15°C. Keep Cell Basement Membrane 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:

1. Thaw Cell Basement Membrane in the refrigerator (2°C to 8°C), in ice, overnight.
2. Dilute the thawed Cell Basement Membrane to 150 µg/mL by directly adding Cell Basement Membrane in cold DMEM: F12 Medium (ATCC 30-2006) on ice and mix well. Immediately coat each 6 cm dish with 2 mL diluted Cell Basement Membrane.
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

Bacterial and fungal testing: Not detected
Mycoplasma contamination: Not detected
Endotoxin: < 8 EU/mL by LAL assay
Functional tests: Each lot of Cell Basement Membrane 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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Cell Basement Membrane (ATCC ACS-3035)

References

References and other information relating to this material are available at www.atcc.org.

Warranty

The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

Disclaimers

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use. Any proposed commercial use is prohibited without a license from ATCC.

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 or complete and the customer bears the sole responsibility

of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with or arising out of the customer's use of the product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at www.atcc.org.

Copyright and Trademark Information

Revision

This information on this document was last updated on 2022-10-22

Contact Information

ATCC
10801 University Boulevard

Cell Basement Membrane

ACS-3035

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor