Corning® Matrigel® Basement Membrane Matrix for 3D Culture *In Vitro*

Protocol

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Corning® Matrigel® basement membrane matrix is a soluble basement membrane extract of the Engelbreth-Holm-Swarm (EHS) mouse tumor that gels at room temperature to form a genuine reconstituted basement membrane. The major components of Corning Matrigel matrix are laminin (~60%), collagen IV (~30%), entactin (~8%) and heparan sulfate proteoglycan. Growth factors, collagenases, plasminogen activators, and other undefined components have also been reported in Corning Matrigel matrix.

Reagents and Materials

- MDCK cell line (ATCC CCL-34, Canis familiaris kidney cell line, derived from normal tissue)
- Corning Matrigel matrix (Corning Cat. No. 356234)
- MEM (Corning Cat. No. 10-009-CV)
- FBS (Corning Cat. No. 35-015-CV)
- PBS (Corning Cat. No. 21-040-CV)
- 0.25% Trypsin/EDTA (Corning Cat. No. 25-053-Cl)
- 24-well plate (Corning Cat. No. 3524)

Protocol 1. On-top MDCK 3D culture

1. Thaw Matrigel matrix overnight by submerging the vial in ice in a 4°C refrigerator before use. Once Matrigel matrix is thawed, swirl vial to ensure the material is dispersed.
2. Add 200 µL of Matrigel matrix (8 to 11 mg/mL) into each well of a pre-chilled 24-well plate, spread evenly with a pipet tip, and then incubate at 37°C for 30 min. to allow the Matrigel matrix to gel.

   Note: All cultureware or media coming in contact with Matrigel matrix should be pre-chilled/ice-cold. Keep Matrigel matrix on ice during the entire process and do not overdry the Matrigel matrix during the gel process.
3. Wash the MDCK cells once with PBS. Trypsinize the cells to make a single-cell suspension, and then pellet the cells through centrifugation at 125 x *g* for 5 min. at room temperature (RT).

   Note: Use cells that are healthy and not more than 85% confluent. MDCK cells tend to form cell clumps; therefore, it is often necessary to pipet them vigorously to obtain a single cell suspension.
4. Re-suspend the cells with MDCK complete medium (MEM + 10% FBS) to adjust the final cell density to 3 x 10^5 cells/mL, plate 250 µL prepared cell suspension into each well of the pre-coated 24-well plate, and then incubate at 37°C for 30 min.

   Note: The number of cells may need optimization depending on the growth properties of the cell line.
5. Chill the MDCK complete medium on ice and add Matrigel matrix to 10% of the final volume (final concentration: 0.8 to 1.1 mg/mL). Gently add 250 µL of Matrigel matrix medium mixture to the plated culture.

   Note: Medium must be thoroughly chilled before the addition of Matrigel matrix to ensure homogenous mixing and even deposition of Matrigel matrix onto cells in culture. Pipet the Matrigel matrix medium mixture down the side of the well to avoid disturbance of the cells or Matrigel Matrix.
6. Continuously culture for 4 to 7 days and change Matrigel matrix medium mixture every 2 days.
7. Immunostaining of 3D cultures and observing cell morphology with confocal microscopy is recommended.

Protocol 2. Embedded MDCK 3D culture

1. Thaw Corning® Matrigel® basement membrane matrix overnight by submerging the vial in ice in a 4°C refrigerator before use. Once Matrigel matrix is thawed, swirl vial to ensure the material is dispersed.
2. On Day 0, dilute the Matrigel matrix to 5 mg/mL with ice-cold MDCK complete cell culture medium (MEM + 10% FBS).

   Note: Keep all cultureware and reagents coming in contact with Matrigel matrix on ice during the entire operation process.
3. Using pre-chilled tips, coat the pre-chilled 24-well plate by adding 100 µL of Matrigel matrix (5 mg/mL) into each well, spread evenly with a pipet tip, and incubate at 37°C for 30 min. to form gel.

   Note: Do not overdry the Matrigel matrix during the gel process.
4. Trypsinize the MDCK cells from a monolayer to make a single-cell suspension, and then pellet the cells through centrifugation at 125 x *g* for 5 min. at room temperature (RT).

   Note: Use cells that are healthy and not more than 85% confluent. MDCK cells tend to form cell clumps; therefore, it is often necessary to pipet them vigorously to obtain a single cell suspension.
5. Re-suspend the cells with MDCK complete medium and adjust the cell density to 5×10^6 cells/mL. Add 30 µL prepared cell suspension to 270 µL Matrigel matrix solution (5 mg/mL) which is kept on ice for a final cell density is 5×10^5 cells/mL. Incubate the plate at 37°C for 30 to 45 min.

   Note: The volume of the cells should not be over 10% of the Matrigel solution to ensure the diluted Matrigel solution can polymerize properly into a matrix. The number of cells may need optimization depending on the growth properties of the cell line.
6. Gently add 500 µL MDCK complete media to each well.
7. Keep culture for 8 to 10 days and change medium every 2 days.
8. Immunostaining of 3D cultures and observing cell morphology with confocal microscopy are recommended.

References

1. Genee Y Lee, et al., Nature Methods. 4(4):359-365 (2007).
1. Natalie Elia, et al., Curr Protoc Cell Biol. Chapter 4:Unit 4.22 (2009).

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