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Expansify™ Technical Note - Extracellular Matrix (ECM) Coating of Expansify™ Plates

Based on internally validated coating conditions and representative experimental data generated using Expansify™ plates.

Purpose

This technical note provides representative conditions for coating Expansify™ plates with extracellular matrix (ECM) proteins prior to adherent cell culture. The guidance is based on internally generated experimental data and includes representative coating concentrations, incubation conditions, and handling considerations for preparing the silicone culture surface. The conditions described are intended to provide a starting point for ECM-coated Expansify™ workflows and may require optimization based on the selected ECM protein, cell type, and intended application. Cell-specific culture performance following ECM coating is described in the corresponding Expansify™ Cell Culture Methods documents.

Materials

Prior to use, review the Expansify™ Quick Start Guide and instructional video series in the resources section of the XDemics website.

- Expansify™ 24-Well Plate (Cat. No. 24W-R-01-01) or Expansify™ 1-Well Plate (Cat. No. 01W-R-01-01).
- Extracellular matrix (ECM) coating reagent appropriate for the intended cell type and application, such as vitronectin, fibronectin, laminin, or another cell-attachment substrate. Vitronectin is used as the representative ECM coating reagent in the conditions described in this technical note.
- Phosphate-buffered saline (PBS), Ca^{2+}/Mg^{2+}-free (Gibco, Cat. No. 10-010-023)
- Centrifuge equipped with a rotor suitable for Expansify™ well plates.
- Incubation conditions appropriate for the selected ECM coating workflow, typically 4°C.

  NOTE: Equivalent products from other manufacturers may be substituted for the ECM coating reagent and Ca^{2+}/Mg^{2+}-free PBS listed above. ECM concentration, coating buffer, and incubation conditions should be selected according to the specific ECM protein, cell type, and intended application. Ca^{2+}/Mg^{2+}-free PBS was used as the standard coating buffer under XDemics-evaluated conditions. Certain ECM reagents require specific buffers for dilution or preparation. When specified, use the buffer recommended by the ECM manufacturer rather than Ca^{2+}/Mg^{2+}-free PBS.

Experimental Procedure

Day -1: ECM Coating

1. Prepare the vitronectin coating solution at a concentration appropriate for the cell type:
    a. **Immortalized cell lines:** A vitronectin concentration of 2–5 µg/mL is recommended as a starting range based on XDemics-evaluated conditions.
    b. **Primary cells:** 10 µg/mL vitronectin is recommended as a starting concentration.
    NOTE: These concentration recommendations are based on XDemics testing with vitronectin. Appropriate coating concentrations may differ for other ECM proteins and should be independently optimized and validated for the selected ECM, cell type, and application.
    NOTE: Under XDemics-evaluated conditions using RoosterBio MSCs and RoosterBio culture medium, truncated vitronectin supported robust cell attachment and growth at 2, 5, and 10 µg/mL following coating incubations of 2 hours at room temperature, 4 hours at room temperature, 16 hours at 4°C, and 24 hours at 4°C. These results are specific to the cell source, media system, coating reagent, and culture conditions evaluated and may vary with other cell types, media, ECM formulations, or applications.
2. Add the coating solution according to the Expansify™ format:
    a. **Expansify™ 24-Well Plate:** Add 1 mL per well.
    b. **Expansify™ 1-Well Plate:** Add 35 mL per plate.
    NOTE: The volumes listed above represent the minimum recommended coating volumes for each Expansify™ format. Larger coating volumes may be used if desired, provided the culture surface remains fully covered during the coating period.
3. Seal the plate with clean Parafilm.
4. Centrifuge the plate at 200 × g for 1 minute, using an appropriate Expansify™ plate or deep-well plate as a counterbalance. This step is required before proceeding to the next step.
5. Remove the plate from the centrifuge and confirm that the coating solution has uniformly wetted the culture surface.
6. Incubate at 4°C for approximately 24 hours to allow adsorption of the ECM protein onto the silicone culture surface.
    NOTE: ECM-coated Expansify™ plates have been used up to 3–4 days after coating under XDemics-evaluated conditions when maintained at 4°C with the coating solution retained in the plate until use.
7. Following incubation, remove the plate from 4°C storage and allow it to equilibrate to room temperature for at least 30 minutes before subsequent cell culture preparation.
8. Aspirate the ECM coating solution before subsequent cell culture preparation. Rinsing the coated surface is optional and is not required under XDemics-evaluated conditions. Do not allow the coated culture surface to dry. Following aspiration, immediately add

complete culture medium or proceed with the appropriate application-specific preparation step.

ECM Selection, Optimization, and Performance Considerations

ECM coating conditions should be optimized for the specific attachment substrate, cell type, and intended application. Parameters such as coating concentration, buffer composition, incubation temperature, and incubation duration may require adjustment because different ECM proteins interact differently with the silicone culture surface and support cell attachment through distinct biological mechanisms.

Vitronectin, fibronectin, and laminin coatings have been validated for use with Expansify™ cultureware under XDemics-evaluated conditions. For applications requiring other ECM proteins or attachment substrates, please contact the XDemics Technical Support team for guidance prior to use.

Representative XDemics-Evaluated ECM Conditions:

Truncated vitronectin: Under XDemics-evaluated conditions using RoosterBio MSCs and RoosterBio culture medium, truncated vitronectin at 2, 5, and 10 µg/mL supported robust attachment and growth across 2-hour and 4-hour room-temperature coating incubations and 16-hour and 24-hour coating incubations at 4°C.

Laminin: In XDemics testing, laminin required a higher coating concentration to support effective attachment on the Expansify™ silicone culture surface. Successful performance was observed at approximately 10 µg/mL or greater following a 24-hour coating incubation at 4°C.

BioLaminin: BioLaminin demonstrated strong performance at 10 µg/mL following a 24-hour coating incubation at 4°C under XDemics-evaluated conditions.

NOTE: These conditions are intended as experimentally supported starting points. Optimal ECM concentration and incubation conditions may vary with ECM formulation, manufacturer, cell type, cell source, culture medium, and intended application.

Not all surface-coating reagents are recommended for use with Expansify™ cultureware. In particular, **poly-D-lysine (PDL) is not recommended** as a stand-alone coating. PDL is a synthetic polycation rather than an extracellular matrix protein and promotes cell attachment primarily through electrostatic interactions with the culture surface. This mechanism may not provide the same stable, biologically relevant attachment environment as ECM proteins on the gas-permeable silicone membrane. Where PDL or other non-ECM attachment substrates are required for a specific application, compatibility and coating performance should be independently evaluated before routine use.

Cell-specific performance data for validated ECM coatings are provided in the corresponding **Expansify™ Cell Culture Methods** documents. These application-specific guides describe the coating conditions and culture performance observed for individual adherent cell types under XDemics-evaluated conditions.