

Coating Mimetix® Scaffold with Basement Membrane Extract


Introduction:

For some cell types and applications it is desirable to coat the Mimetix scaffold with Basement Membrane Extract to enhance attachment. The protocol for use is similar to that for 2D culture, though quantities may be increased. The following standard protocol can be varied according to application.

Method:

1. Precondition Mimetix scaffold for coating by: treating with 20% ethanol; soaking for 5 minutes; aspirating carefully to remove the ethanol without touching the scaffold; washing twice with PBS (see Mimetix 'guidelines for use' for volumes). Leave in the second PBS wash until ready to apply the coating.
2. Dilute Basement Membrane Extract to a concentration of 0.8 mg/ml (1 in 10 dilution) using appropriate cell culture media. Handle the reagents on ice, using pre-chilled pipette tips to perform the dilution and subsequent application onto the Mimetix scaffold.
3. Aspirate the second PBS wash from the Mimetix scaffold and carefully pipette the diluted Basement Membrane Extract solution onto each disc. Add sufficient amount of solution to cover the entire surface area (200-350 μl per cm^2). Replace plate lids and leave to stand for 1-2 hours at room temperature.
4. Remove excess fluid. Aspirate to remove any residual coating agent from the bottom of the wells. If using the 12-well plate format, tilt the plate and gently aspirate any excess fluid from the edge of the wells.
5. Prepare cells for seeding in the appropriate culture media and seed directly on the wet Basement Membrane Extract coated Mimetix scaffold (see cell seeding protocol). Allow the cells to settle for 30-90 minutes in an incubator (5 % CO_2 , 37 °C) before adding more media.

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