Collagen Coating for Tissue Culture

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Method Article

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Abstract

This protocol is intended for collagen coating of tissue culture dishes and Transwell® multiple well plates. The attached methods document is a formal version of the information included here.

Disclaimer: The information presented here has been reviewed and approved for publication by the US Environ do not necessarily represent Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendations for use.

Introduction

Reagents

Advanced BioMatrix PureCol® bovine collagen solution, Type I, 3.0 mg/mL (#5005-100ML)

Cell culture grade water (Corning #25-055-CM)

Dulbecco's phosphate buffered saline (DPBS, 1X, Gibco #14190-144)

Equipment

Biosafety cabinet

Humidified tissue culture incubator with 5% CO_2

Tissue culture dishes

-100mm dishes (TPP #93100)

-150mm dishes (TPP #93150)

Costar® clear TC-treated multiple well plates

-6 well plates (Corning #3516)

-12 well plates (Corning #3512)

Transwell® 0.4µm pore polyester membrane inserts

-24mm inserts (Corning #3450)

-12mm inserts (Corning #3460)

50 mL conical tubes

Sterile 150 mL, 250 mL, 500 mL, and/or 1 L bottles (depending on number and size of plates/inserts being coated)

Pipetaid

Serological pipettes

Pipettes

Filter tips (low retention)

Procedure

1. Dilute PureCol® collagen (3.0 mg/mL) to 50 µg/mL (1:60) in cell culture grade water in a sterile 50 mL conical tube or appropriately sized bottle.

a. **NOTE:** PureCol collagen needs to be kept on ice.

2. Swirl contents gently, but thoroughly, until material is completely mixed.

3. Add appropriate amount of the mixture to the tissue culture dish, well, or insert in the following volumes and swirl to ensure the entire surface is coated:

a. Tissue culture dishes

i. 7 mL per 100mm dish

ii. 15 mL per 150mm dish

b. 6 well plates

i. 1.0 mL per 24mm insert

ii. 1.5 mL per well

c. 12 well plates

i. 250 µL per 12mm insert

ii. 750 µL per well

4. Replace the dish lid and incubate in a biosafety cabinet (or another covered, dry location) at room temperature for 2 hours.

5. Carefully aspirate the collagen solution.

6. Rinse coated surfaces with the following volumes of sterile PBS:

a. Tissue culture dishes

-7 mL per 100mm dish

-15 mL per 150mm dish

b. 6 well plates

-1.0 mL per 24mm insert

-1.5 mL per well

c. 12 well plates

-250 µL per 12mm insert

-750 µL per well

7. Carefully aspirate the wash. Allow residual wash to pool by tilting the plates to facilitate aspirating as much of the wash as possible.

8. Air dry dishes and plates in biosafety cabinet, laid out flat with the lids cracked slightly open.

9. Label with the date in green permanent marker. Repackage dishes and plates in their original bags and store in plastic bins at 4°C.

Troubleshooting

Time Taken

Anticipated Results

References

https://www.advancedbiomatrix.com/wp-content/uploads/2016/12/5005-DFU-PureCol-Solution-Rev-08.pdf

Acknowledgements

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- McCulloughMethodsCollagenCoating.docx