Research Square

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FITC-Dextran Trans-Epithelial Permeability Assay

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Method Article

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Abstract

This protocol describes the use of the Fluorescein isothiocyanate (FITC)–dextran trans-epithelial permeability assay to quantify the effects of chemicals on epithelial cell monolayer permeability.

The attached methods document is a formal version of the information included here.

Disclaimer: The contents of this article have been reviewed by the US Environmental Protection Agency and approved for publication and do not necessarily represent Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendations for use.

Introduction

Reagents

FITC-Dextran average molecular weight 20 kDa (Sigma #FD20S)

MEM w/out phenol red (ThermoFisher Scientific #51200038)

Equipment

Humidified tissue culture incubator with 5% CO_2

50 mL conical tubes

Ice bucket

Pipetmen

Balance

Polarstar Plate Reader

Corning™ 96-Well Clear Bottom Black Polystyrene Microplates

Filter tips (low retention)

Procedure

Preparation of FITC-Dextran Solution

Resuspend FITC-Dextran in phenol-red free MEM warmed to 37 ^{o}C to a concentration of 1 mg/mL in a sterile environment. Protect solution from light.

Assay Procedure

1. Prepare triplicate 12-well collagen coated inserts for monolayer-free positive control within multi-well plate set-up.

2. Expose test samples with compound of interest.

3. Transfer and blot Transwell® inserts containing exposed cells to new multi-well plate and add 800 µL phenol red-free MEM to basolateral compartment.

4. Aspirate apical compartment media and replace with 250 µL FITC-Dextran suspension.

5. Protect from light at room temperature for 20 minutes.

6. End FITC-Dextran reaction by transferring Transwell® inserts with FITC-Dextran suspension to new multi-well plate.

7. Collect FITC-Dextran suspension basolateral media in 1.5 mL Eppendorf tubes and vortex.

8. Transfer 100 µL aliquots from each Eppendorf tube containing FITC-Dextran basolateral media to Corning™ 96-Well Clear Bottom Black Polystyrene Microplates in triplicate.

9. Transfer 100 µL of phenol-red free MEM to an additional three wells to serve as the blank control.

10. Measure fluorescence intensity on the Polarstar Plate Reader using 490/520 nm excitation/emission maxima.

a. **Note:** Before measuring fluorescence, use the Polarstar's "Adjust Gain" setting to adjust all your samples to the well with the highest fluorescence.

11. Export data to Excel for analysis.

Troubleshooting

Time Taken

Anticipated Results

References

Acknowledgements

Supplementary Files

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

- McCulloughMethodsFITCDextranPermeabilityAssay.docx