Endothelial paracellular permeability assay

Laura Ruiz Remolina

Abstract

The human umbilical vein endothelial cell line EA.hy926 (ATCC; Mannasas, VA, USA) was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS) in an atmosphere of 5% CO2/95% air and 37°C.

EA.hy926 cells (10^5cells/plate) were seeded in transwell inserts (Corning® Biocoat™ Cell Culture Inserts Collagen, Type I Rat Tail, 24-Well, 3 µm; Corning, NY, USA) and were cultured to confluence at 24 h. The cells were starved overnight and then activated with LPS (1 µg/ml) for 4 h. Upper chamber media, containing LPS (1 µg/ml) and soluble endoglin (500 ng/ml) for their respective treatments, were replaced with FITC-Dextran (40 kDa) at 1 mg/ml in DMEM. The bottom chambers were also replaced with DMEM. After 24 h at 37°C the inserts were removed, and the amount of fluorescence in the bottom chambers was measured using a fluorescence plate reader (Fluoroskan Ascent FL; Thermo Electron Corporation, Waltham, MA, USA).

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Guidelines

Avoid repeated freeze-thaw cycles

Materials

EA.hy926 by ATCC
Transwell Biocoat Cell culture Insters by Corning
LPS L3129 by Sigma-aldrich
✓ Recombinant human endoglin 1097-EN-025 by Contributed by users
DMEM 41966 by Thermo Fisher Scientific
✓ Fluoroskan Ascent FL by Contributed by users

Protocol

Culture the cell line EA.hy926 (ATCC; Mannasas, VA, USA) was cultured in DMEM) supplemented with 10% fetal calf serum (FCS) in an atmosphere of 5% CO2/95% air and 37°C.

Step 1.

Seed EA.hy926 cells (105cells/plate) in transwell inserts (Corning® BiocoatTM Cell Culture Inserts Collagen, Type I Rat Tail, 24-Well, 3 µm; Corning, NY, USA) and cultured it to confluence at 24 h.

Step 2.

Starve the cells overnight and activate it with LPS (1 µg/ml) for 4 h.

Step 3.

Upper chamber media, containing LPS (1 µg/ml) and soluble endoglin (500 ng/ml) for their respective treatments, were replaced with FITC-Dextran (40 kDa) at 1 mg/ml in DMEM.

Step 4.

Replace the bottom chambers of the transwell with DMEM.

Step 5.

After 24 h at 37°C remove the inserts and measure the amount of fluorescence in the bottom chambers using a fluorescence plate reader (Fluoroskan Ascent FL; Thermo Electron Corporation, Waltham, MA, USA).

Step 6.