For Research Use Only

Endothelial Transwell Permeability Assay Kit (24 SPL PC inserts, 100 assays)

Catalog Number: CB6929

Introduction

The microvascular endothelial cell monolayer localized at the critical interface between the blood and vessel wall has the vital functions of regulating tissue fluid balance and supplying the essential nutrients needed for the survival of the organism. The endothelial cells are able to dynamically regulate its paracellular and transcellular pathways for transport of plasma proteins, solutes, and liquid. The endothelial barrier is a well-regulated structure, which maintains a minimal and selective permeability to fluid and molecules under normal physiological conditions. Vascular leakage is an important feature in several diseases, such as septic shock, inflammation, disruption of blood-brain barrier (BBB)-related neurological disorders, viral hemorrhagic fever, cancer and ischemia-reperfusion injuries. It is emergent to develop a highly sensitive and reliable assay to measure endothelial permeability.

A simple, reproducible, sensitive, quantitative transwell permeability assay is developed to measure endothelial barrier function for wide analyses of multiple types of endothelial permeability *in vitro*. The assay will be performed by a spectrometer-based absorbance reader (ELISA plate reader) at 450 nm within one hour post endothelial treatment. The assay kit is suitable for primary endothelial cells and immortal cell lines from multiple species.

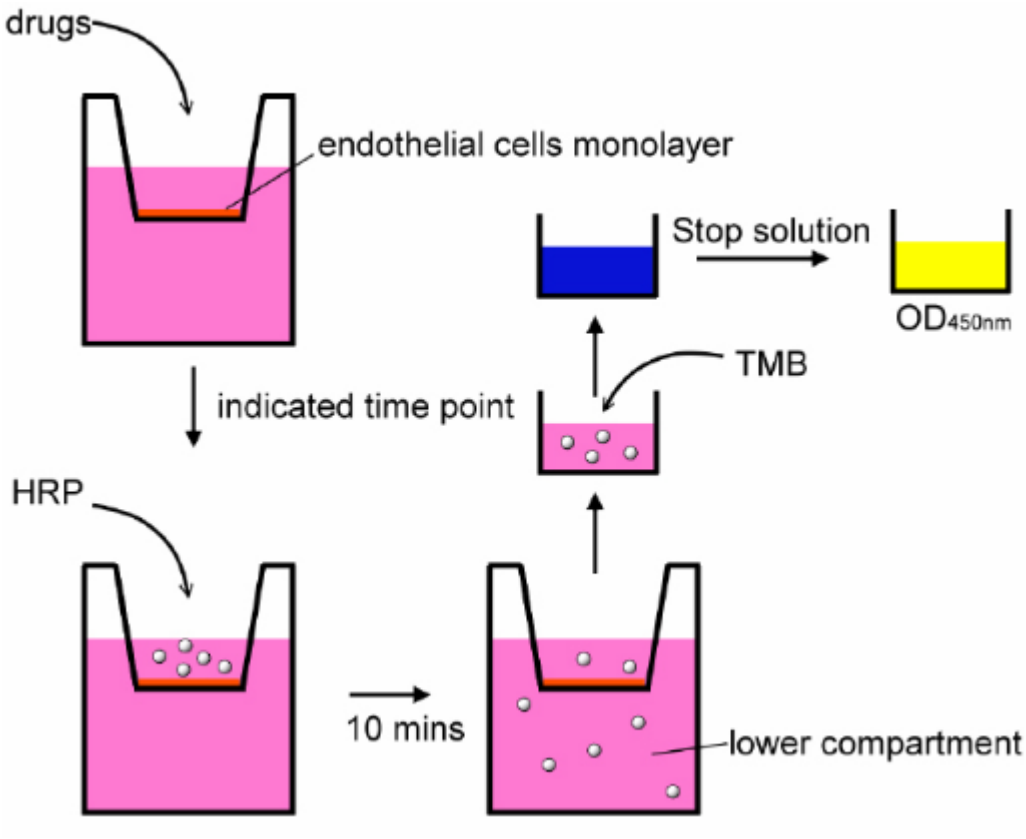
Storage Temperature: 4 °C.

Kit Components (for 100 assays in 96 well plates):
A: SPL PC membrane, 6.5 mm, 0.4 µm pore, white, sterile 24 inserts in a 24-well cell culture plate.
B: A 96-well ELISA plate (clear polystyrene wells, flat bottom).
C: Streptavidin-HRP solution (150 µl).
D: TMB substrate solution (5 ml).
E: Stop solution (5 ml)

Equipment Required but Not Provided:

Spectrophotometric multi-well plate reader or Beckman spectrophotometer

A schematic outline procedure



Procedure

All samples and standards should be run in triplicate.

1. Add 1 ml endothelial growth medium in the experiment wells of 24-well plate.
2. About 1~5 x 10^5 endothelial cells with or without transfection were seeded on the 6.5 mm transwell insert membrane (0.4 µl) in 200~400 µl endothelial growth medium.
3. Transfer the inserts to the wells containing 1 ml of growth medium using a sterilized forceps and cover the plate.
4. Incubate the plate in a cell culture incubator for 2 to 4 days until cells grow by 100% confluence. To check the cell confluence, move the inserts to the empty wells with a sterilized forceps, and wait for 5 minutes to check whether the medium from the upper inserts leaks into the wells of 24-well plate. If there is no leak, then proceed to the next steps. Otherwise, the cells need to grow for days till the cells grow a confluent monolayer.
5. Endothelial cells were starved in a serum-free or 0.1% serum medium for a proper time according to individual experiment purpose.
6. Add the desired stimulus (TNF-α, IL-1β, thrombin, LPS, etc.) and 5 µl of streptavidin-HRP to the 300 µl fresh serum-free medium in the top insert chambers.
7. Add 1 ml serum free medium in the wells of a new 24-well cell culture plate and transfer the inserts in to incubate in the 37 °C incubator for a proper treatment time. This assay works best when the cells are treated between 30 min to 24 hours.
8. Transfer 20 µl of media from the lower chamber to the wells of 96-well ELISA assay plate. Each sample would be at least aliquoted in triplicate. No treatment or HRP-free medium were considered as negative control.
9. Add 50 µl TMB substrate into each well of 96-well plate, incubate in a shaker for a couple of minutes at room temperature until the blue color occurred.
10. Add 50 µl stop solution into the wells of 96-well plate, and the blue color will become yellow. Measure the spectrum absorption at 450 nm using an ELISA plate reader. Data expressed as A450 absorption readings are considered a relative permeability.