QCM™ Leukocyte Transendothelial Migration Assay - Colorimetric

Sufficient for analysis of 24 samples

Cat. No. ECM557

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

Leukocytes patrol the vascular system. They must migrate across endothelial barriers in order to recruit to sites of inflammation. This process involves a multistep cascade consisting of leukocyte rolling, adhesion, and transmigration. A quantitative assay for leukocyte transendothelial migration has been described using a modified Boyden chamber system (Roth et al. 1995, Ding et al. 2000). The Boyden chamber system is a two chamber system with a porous membrane providing an interface between these two chambers. Endothelial cells are cultured on top of the porous membrane that is coated with an extracelluar matrix (ECM) protein. Once a confluent layer of cells is established, leukocytes are then added onto the endothelial monolayer. Leukocyte migration across the endothelium is determined by measuring the number of cells that migrate between the endothelial cells, through the porous membrane, to the lower chamber.

Millipore's Colorimetric QCM™ Leukocyte Transendothelial Cell Migration Assay provides an efficient model to analyze the ability of leukocytes to migrate through the endothelium. The assay is designed with a 3 µm pore size cell culture insert, appropriate for most leukocyte migration. The upper side of the cell culture insert is coated with fibronectin to support the optimal attachment and growth of endothelial cells. The assay allows investigators to evaluate the effects of various factors that influence the transendothelial process.

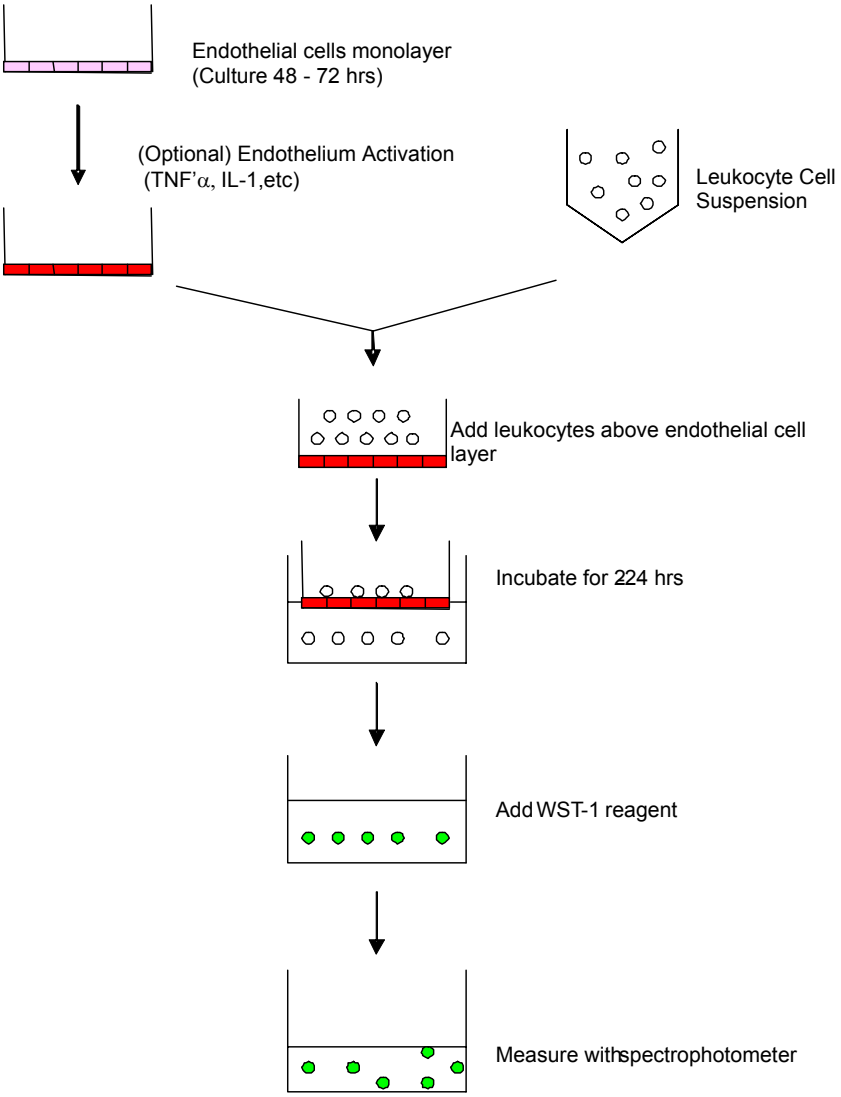
Precoated cell culture inserts are provided in the Millipore QCM Leukocyte Transendothelial Cell Migration Assay to significantly reduce assay time. Additionally, the assay allows quantitative analysis of leukocyte migration. Following incubation of leukocytes with the endothelial cell layer, migratory cells are stained and quantified with WST-1 reagent and measured using a spectrophotometer. Absorbance correlates with cell migration.

Each kit offers a sufficient number of chambers to evaluate 24 samples.

In addition to this QCM assay system, Millipore offers:

- QCM Tumor Cell Transendothelial Cell Migration Assay–Colorimetric (Cat. No. ECM558)
- Endothelial Cell Adhesion Assay (Cat. No. ECM645)
- QCM 3 µm Endothelial Cell Migration Assay-Fibronectin, Colorimetric (Cat. No. ECM200)
- QCM 3 µm Endothelial Cell Migration Assay-Fibronectin, Fluorometric (Cat. No. ECM201)
- *In Vitro* Angiogenesis Assay Kit (Cat. No. ECM625)
- Fibrin *In Vitro* Angiogenesis Assay Kit (Cat. No. ECM630)
- Endothelial Cell Adhesion Assay Kit (Cat. No. ECM645)
- *In Vitro* Vascular Permeability Assay (Cat. No. ECM640)
- Tumor Necrosis Factor-α, recombinant human (Cat. No. GF023)
- EndoGRO HUVEC (Cat. No. SCCE001)
- EndoGRO-LS complete Media Kit (Cat. No. SCME001)

Assay Principle



Kit Components

1. Migration Test Plate: (Part No. CS202626) Two 24-well culture plates, each containing 12 human fibronectin-coated cell culture inserts (3 μm pore size), sufficient for the evaluation of 24 test samples
2. TNFα: (Part No. 2004167) One tube - 20 μL at 0.1 mg/mL
3. Cell Stain Solution: (Part No. 20294) One vial - 10 mL
4. WST-1 Reagent: (Part No. CS201370) One vial.
5. Electro Coupling Solution: (Part No. CS201382) One vial - 5 mL.
6. 96 well Stain Quantitation Plate: (Part No. 2005870) One plate.
7. Forceps: (Part No. 10203) 1 pair

***Caution**: Cell Stain Solution contains a small amount of crystal violet, which is toxic if swallowed or inhaled, and may cause irritation to the eyes, respiratory system, and skin. Handle with caution.*

Materials Not Supplied

1. Endothelial cells (for example: HUVECs, Cat No. SCCE001)
2. Endothelial cell culture medium (Cat. No. SCME001)
3. Leukocytes and cell culture medium
4. Harvesting buffer: Millipore's cell detachment solution, Accutase™ (Cat. No. SCR005), EDTA or trypsin-based cell detachment buffer, or other cell detachment formulations as optimized by individual investigators can also be used.
5. **Serum-free** medium, such as RPMI-1640, DMEM (Cat No. ES-101-B), MEM, etc. containing 0.5% BSA (Cat No. 82-046-4), Pen/Strep (Cat No. TMS-AB2-C).
6. Sterile PBS (Cat No. BSS-1006-B) to wash cells.

7. Distilled water (Cat No. TMS-006-B)
8. (Optional) Chemoattractant or pharmacological agent for addition to culture medium.
9. Low speed centrifuge and tubes for cell harvesting.
10. CO_2 incubator appropriate for subject cells.
11. Microplate reader (420 - 480 nm detection) or a spectrophotometer

Storage

The kit is packaged in two individual boxes. ECM557-1 contains components that upon arrival should be stored at 2° to 8°C. ECM557-2 contains TNFα , WST-1, and Electro Coupling Solution that upon arrival should be stored at -20°C. Please refer to kit label for expiration date.

Assay Instructions

Cell Harvesting

Prepare subject cells for investigation as desired. The following procedure is a suggested protocol for HUVECs and may be optimized to suit individual cell types.

1. HUVECs are maintained in Endothelial cell growth media supplied with serum and growth factors (see Materials Not Supplied section). We recommend using a lower passage of HUVECs (passage 1-8).
2. Wash cells once with sterile Harvesting buffer (see Materials Not Supplied).
3. Add 3 mL Harvesting Buffer per 100 mm dish and incubate at 37°C for 5 minutes.
4. Pipet cells off dish gently and add to 10 mL cell culture medium.
5. Centrifuge cells gently to pellet (300 x g for 5 minutes).
6. Gently resuspend pellet in 10 mL cell culture medium.

Transendothelial Migration Assay

1. Add $1x10^5$ endothelial cells in 250 μL endothelial cell culture medium to each insert. (Important: Leave the lower chamber of the 24 well culture plate empty. It will help to reduce background signal.)
2. Culture the cells for 48-72 hrs until the endothelial cells form a monolayer. (If desired, verify the integrity of the endothelial cell monolayer. Stain one chamber of endothelial cells for 10 min. with 250 μL of the Cell Stain Solution using provided in the kit.. Rinse twice with distilled water and tap dry. The cells must be >95% confluent when viewed under a microscope (see Figure 1 on pg. 9).
3. (Optional) Activate endothelial cell monolayer with 20 ng/mL of recombinant human TNFα for 4-18 hrs.
4. Harvest leukocytes and wash once with serum-free medium containing 0.5% BSA. Resuspend the cells in serum-free medium. Compounds that stimulate or inhibit tumor cell migration (cytokines, pharmacological agents, etc) may be added to the cell suspension.
5. Carefully remove the endothelial cell culture medium from the cell culture insert (Do not disturb the endothelial monolayer.). Transfer the inserts to new wells containing 300 μL of serum-free leukocyte growth medium containing 0.5% BSA, in the presence or absence of chemoattractant.
6. Add $2x10^5$ leukocytes in 250 μL cell culture medium to the upper well of the inserts.
7. Incubate 2-24 hrs in the cell incubator.

Staining Procedure

The following steps may be performed in a non-sterile environment.

1. Using the included forceps, grasp each Millicell insert and gently remove it from the plate and discard.
2. Transfer 200 µL of the 300 µL solution from the lower chamber (containing migrated leukocytes) to the included 96-well plate.
3. Dissolve the lyophilized WST-1 reagent with 5 mL of the Electro Coupling solution.
4. Add 20 µL/ well WST-1 solution to each well.
5. Incubate cells for 0.5-4 hours in standard culture conditions.

   Note: *The appropriate incubation time depends on the individual cell type and cell concentration used.*
6. Shake thoroughly for one minute on a shaker.
7. Measure the absorbance of the treated and untreated samples using a microplate reader at 420 - 480 nm. Use the same amount of culture medium and WST-1 solution in an empty well as a blank position for the microplate reader. Check O.D. every 15-30 minutes for optimal results.

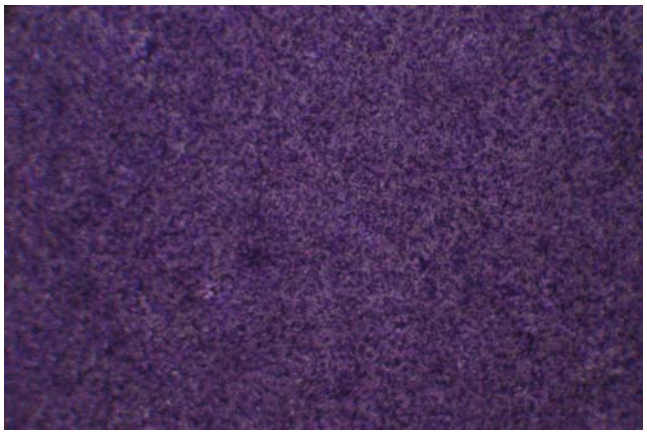
Calculation of Results

Results of the assay may be illustrated graphically using a bar chart to display optical density (OD) values at 420 - 480 nm.

Additional migration may also be induced or inhibited in test wells through the addition of cytokines or other pharmacological agents. Figures 2 and 3 demonstrate typical migration results. One should use the data for reference only. This data should not be used to interpret actual assay results.

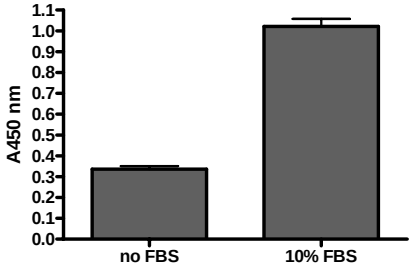
Sample Results

Figure 1.



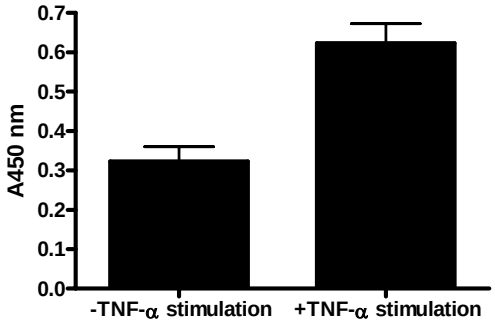
Endothelial Cell Layer. Image depicts a confluent endothelial cell layer after 72 hour incubation on a cell culture insert. Cells are stained with Cell Stain Solution provided in the kit.

Figure 2.



Transendothelial migration of HL-60 cells. HUVECs ($1x10^5$ cells) were grown to confluency for 72 hrs on cell culture inserts. HL-60 cells ($2x10^5$ cells) were added on the endothelial layer and left to migrate for 18 hrs at 37°C. Migrated cells were stained and measured by OD 450 nm according to the Assay Instructions. Data represents the mean $\pm$S.D., n=3.

Figure 3.



Transendothelial migration of HL-60 with TNFα stimulation of HUVECs. HUVECs ($1x10^5$ cells) were grown to confluency for 72 hrs on cell culture inserts. HUVECs were then treated with 20 ng/mL of TNFα for 4 hrs. HL-60 cells ($2x10^5$ cells) were added on the endothelial layer and 10% FBS was used as chemoattractant in the lower chamber. Cells were left to migrate for 4 hrs at 37°C. Migrated cells were stained and measured by OD 450 nm according to the Assay Instructions. Data represents the mean ±S.D., n=3.

References

1. Ding, Z., *et al*. (2000). Regulation of chemokine-induced transendothelial migration of T lymphocytes by endothelial activation: differential effects on naive and memory T cells. *J. Leukoc. Biol*. **67**: 825.

2. Roth, S.J., *et al*. (1995). Characterization of transendothelial chemotaxis of T lymphocytes. *J. Immunol. Methods*. **188**:97.

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