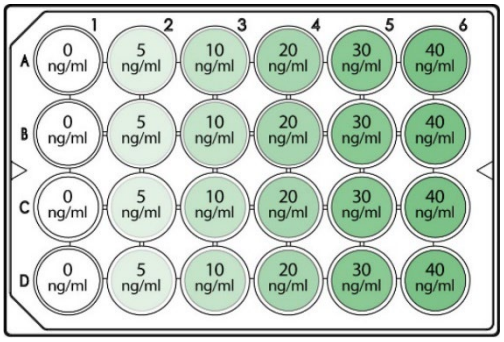
Wound Healing Assay Using the ibidi Culture-Insert 2 Well in a µ-Plate 24 Well



Cell migration studies serve as a cornerstone for dissecting complex physiological and pathological systems. By capturing time-lapse images under a microscope, the wound healing assay allows for the real-time quantification of cell movement, enabling researchers to calculate parameters such as migration velocity, directional persistence, and overall gap closure. This becomes particularly useful for assessing the role of specific genes, signaling pathways, or pharmaceutical agents in modulating cell migration kinetics.

In this Application Note, we outline a detailed protocol for studying MCF-7 breast cancer cell migration using the Culture-Insert 2 Well in a µ-Plate 24 Well ibiTreat. Additionally, we investigate the effect of various concentrations of human epidermal growth factor (hEGF) on cell migration, conducting this experiment in technical quadruplicate for robust data analysis.

The ibidi Culture-Insert 2 Well provides two cell culture reservoirs, each separated by a 500 µm wall. Filling cell suspension in both reservoirs allows cell growth in the designated areas only. Removing the Culture-Insert 2 Well after appropriate cell attachment creates a cell-free gap of approximately 500 µm. Depending on your focus of interest, the wound healing process can either be done by using video microscopy, or by observing images at distinct time points. Measuring the changes in the cell-covered area allows for quantifying the speed of wound closure and provides cell migration characteristics.

Choose from our Culture-Insert 2 Well product variations:

- in µ-Dish 35 mm, high (81176) or in µ-Dish 35 mm, low (80206) for focused studies
- in µ-Plate 24 Well (80242) for high-throughput applications
- Culture-Insert 2 Well for self-insertion into your own vessel (80209)

Please note: Before starting a wound healing assay, empirically determine key parameters such as cell density, medium composition, culture vessel, and concentrations of the bioactive compound. Moreover, it is highly recommended to set up biological and technical control experiments to validate the results. It is essential to conduct a comprehensive literature review before beginning the experiment.

More detailed information is provided in the following PDF documents:

- Application Note 21: “Wound Healing Assay Using the ibidi Culture-Insert 2 Well in a µ-Dish 35 mm ”
- Application Note 30: “Optimizing Wound Healing and Cell Migration Assays”
- Application Note 67: “Data Analysis of Wound Healing and Cell Migration Assays”

1. Materials

1.1. Reagents and Buffers

- MCF-7 (HTB-22, ATCC)
- Cell culture medium: RPMI (R8758, Sigma Aldrich) with 10% FCS (F0804, Sigma Aldrich)
- D-PBS (14190144, Gibco)
- Accutase (A1110501, Gibco)
- Human epidermal growth factor (hEGF) (E9644, Sigma Aldrich)

1.2. Equipment

- Culture-Insert 2 Well 24 ibiTreat (80242, ibidi)
- Sterile tweezers
- Inverted brightfield or phase contrast microscope
- Stage top incubator for live cell imaging (e.g., ibidi Stage Top Incubator Multiwell Plate CO_2/O_2 – Silver Line, 12724, ibidi)
- Standard cell culture equipment (pipettes, sterile working bench, cell culture incubator, culture flasks, cell culture medium, hemocytometer, etc.)

2. Methods

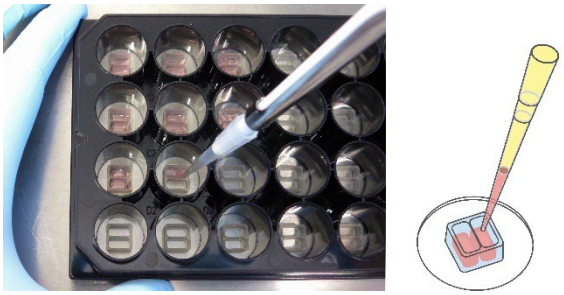
Please read the Instructions before working with the Culture-Insert 2 Well 24 ibiTreat. Perform all steps under sterile conditions. Before starting the experiment, prepare the MCF-7 cells in a standard cell culture flask (e.g., T75) with the cells adherent at the bottom. The cells should be healthy and optimally subconfluent on the day of the experiment.

It is important to work swiftly during the whole procedure so the wells will not dry out.

If not stated otherwise, all given volumes are per well of the Culture-Insert 2 Well, and all incubation steps are at room temperature.

2.1. Cell Cultivation

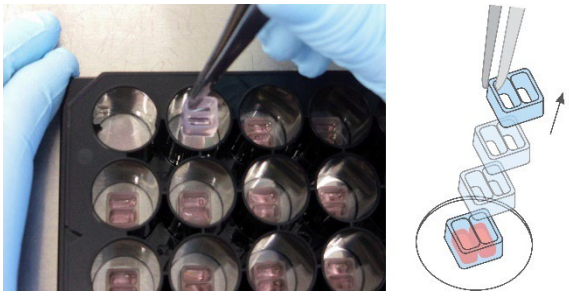
- Treat the cultured MCF-7 cells with Accutase for 1–2 min for detachment.
- Harvest the cell suspension, centrifuge, and dilute it in a low amount of cell culture medium for counting.
- Count the cells and adjust to a final concentration of 5×10^5 cells/ml in cell culture medium.
- Unpack the Culture-Insert 2 Well 24 (hereafter referred to as µ-Plate).
- Apply 70 µl of the MCF-7 cell suspension into each well of the Culture-Insert 2 Well. Avoid shaking the µ-Plate as this will result in an inhomogeneous cell distribution.
- Cover the µ-Plate with the supplied lid.
- Place the µ-Plate into the incubator (37 °C, 5% CO_2) and let the cells attach overnight.



Filling of the Culture-Insert 2 Wells with cell culture medium.

2.2. Gap Formation and Compound Addition

- Examine the cell density via microscopy; if not confluent, return the µ-Plate to the incubator and monitor periodically until confluency has been reached.
- Prepare 1 ml aliquots of hEGF working dilutions in cell culture medium for each designated well, with concentrations of 0, 5, 10, 20, 30, and 40 ng/ml.
- Gently remove the Culture-Inserts by lifting them at one corner with sterile tweezers; check whether the cell layer is still attached to the µ-Plate surface.
- Slowly add 200 µl D-PBS into each well and aspirate to wash away debris and detached cells.
- Slowly add 1 ml of the respective hEGF working dilutions into the designated wells.

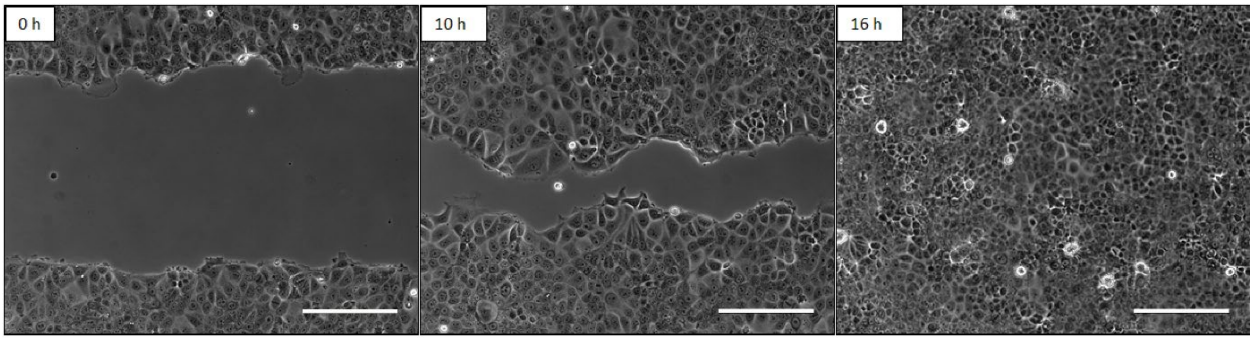


Removing the Culture-Insert 2 Well with sterile tweezers.

2.3. Image Acquisition

More detailed information about optimal image acquisition is provided in Application Note 30: “Optimizing Wound Healing and Cell Migration Assays”.

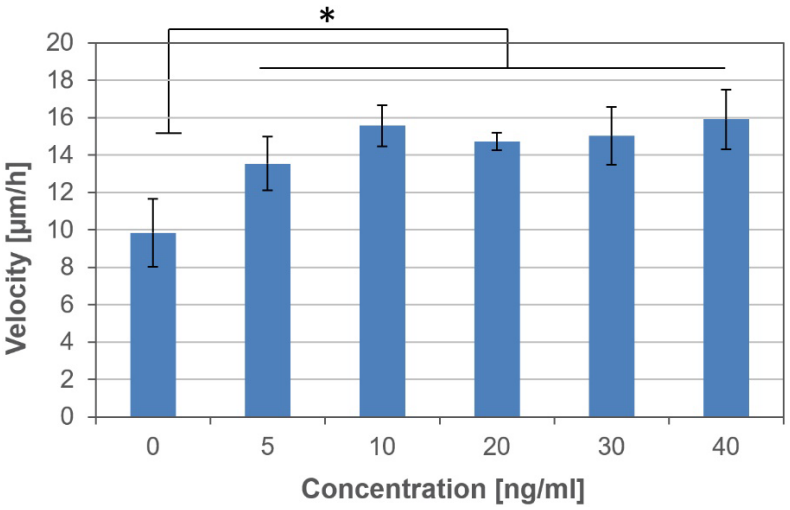
- Place the µ-Plate under a microscope, preferably equipped with automated image acquisition software and an ibidi Stage Top Incubator.
- Use a 4x, 5x, or a 10x objective lense for imaging.
- Define the positions of all wells for live cell imaging. Make sure you have the gap and both cell fronts captured in the image. The orientation of the wound area needs to be horizontal or vertical.
- Start live cell imaging by capturing images every 30 min for 24 h. Note that the timing may need adjustment depending on experimental variables such as cell type, passage number, and medium composition.



Time lapse measurement of a migration assay using MCF-7 cells (scale bar: 200 µm).

2.4. Data Analysis

- To quantify cell migration over the wound area, analyze the cell-covered area in the acquired images at various time points as described in Application Note 67: “Data Analysis of Wound Healing and Cell Migration Assays”.



Influence of different hEGF concentrations on the migration behavior of MCF-7 cells in a wound healing assay. MCF-7 cells were cultured in technical quadruplicate using the Culture-Insert 2 Well 24 and incubated for 24 h. The migration velocity was quantified at 30 min intervals over a 24 h period.