Product Manual

CytoSelect™ 24-Well Wound Healing Assay

Catalog Number

CBA-120	24 assays
CBA-120-5	5 x 24 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

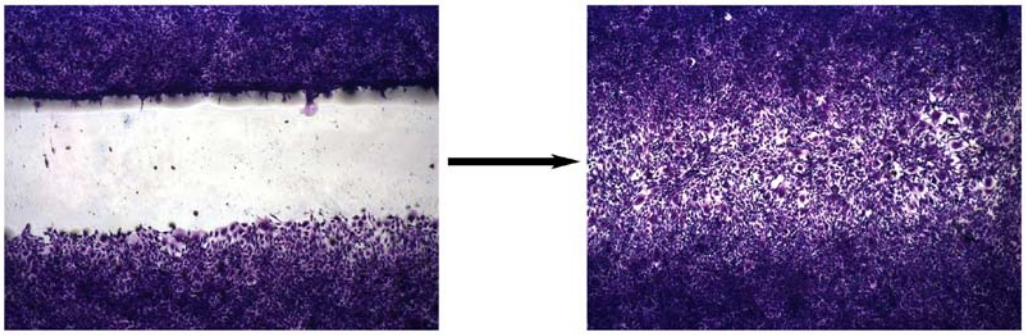
Introduction

Wounded tissue initiates a complex and structured series of events in order to repair the damaged region. These events may include increased vascularization by angiogenic factors, an increase in cell proliferation and extracellular matrix deposition, and infiltration by inflammatory immune cells as part of the process to destroy necrotic tissue. The wound healing process begins as cells polarize toward the wound, initiate protrusion, migrate, and close the wound area. These processes reflect the behavior of individual cells as well as the entire tissue complex.

Wound healing assays have been employed by researchers for years to study cell polarization, tissue matrix remodeling, or estimate cell proliferation and migration rates of different cells and culture conditions. Wound healing assays have been used to study cell polarity and actin cytoskeletal structure regulation through the role of Rho family GTPases, microtubule and Golgi apparatus orientation, the role of p53 in cell migration, as well as other physiological processes. These assays typically involve culturing a confluent cell monolayer and then displacing or destroying a group of cells by scratching a line through the monolayer. The open gap created by this "wound" is then inspected microscopically over time as the cells move in and fill the damaged area. This "healing" effect can take several hours to several days depending on the cell type, conditions, and the surface area of the "wounded" region. The disadvantage of these "scratch wound" assays is the lack of a defined wound surface area, or gap between cells. These wounds are varying sizes and widths, which inhibits consistent results and creates variation from well to well. In addition, the "scratch wound" assay often causes damage to the cells at the edge of the wound, which can prevent cell migration into the wound site and healing.

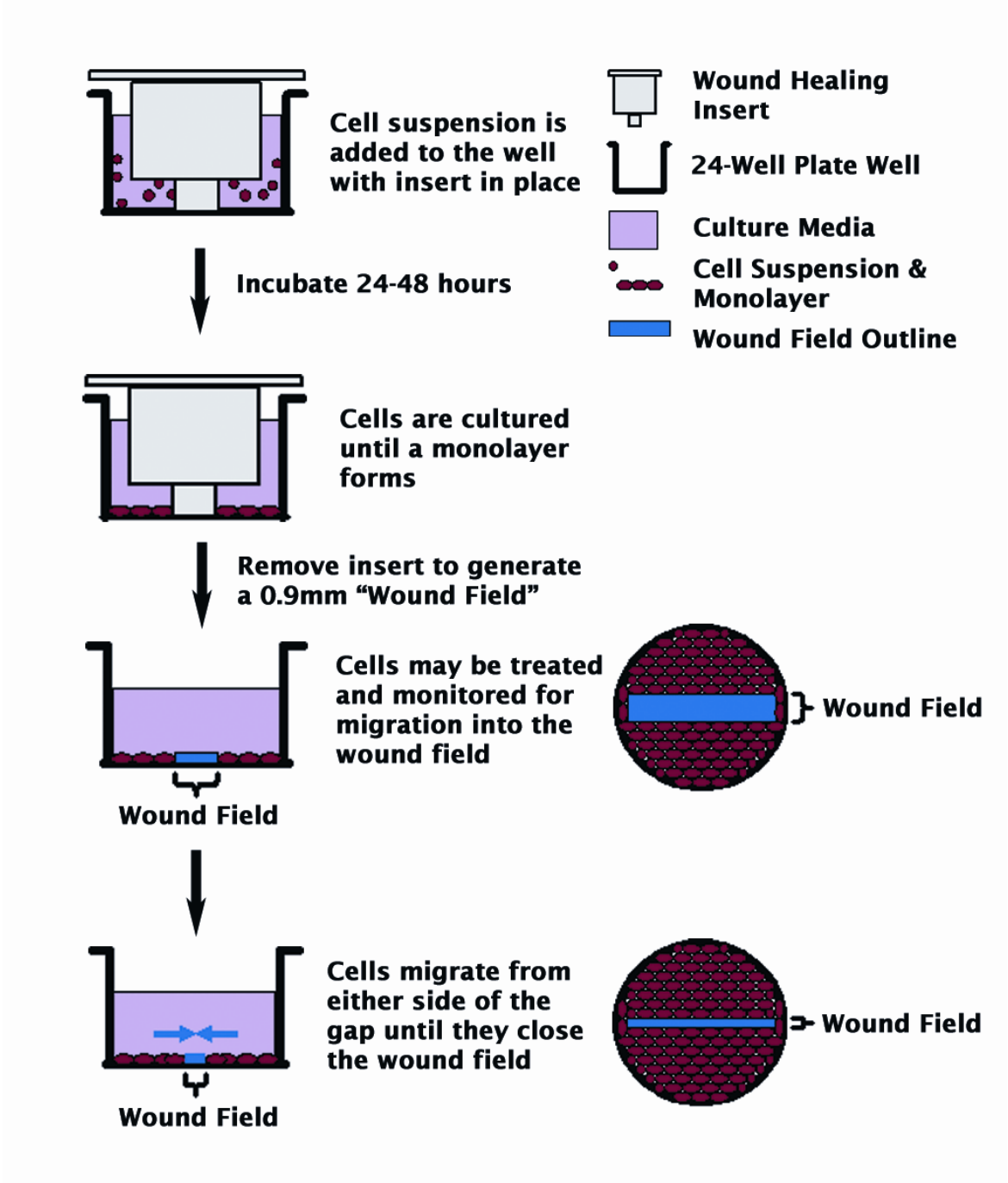
Our CytoSelect™ Wound Healing Assay Kit overcomes this inconsistency by providing proprietary treated inserts that can generate a defined wound field or gap. Cells are cultured until they form a monolayer around the insert. The insert is removed, leaving a precise 0.9 mm open "wound field" between the cells. Cells can be treated and monitored at this point for migration and proliferation into the wound field. Progression of these events can be measured by imaging samples fixed at specific time points or time-lapse microscopy.

Cell Biolabs CytoSelect™ Wound Healing Assay Kit includes proprietary "wound field" inserts to assay the migratory and wound healing characteristics of cells. The kit contains sufficient reagents for the evaluation of 24 samples. The insert is optimal for use with most cell types and experimental conditions. The 0.9 mm wound field generated is compatible for use with most microscopes and imaging systems.



Assay Principle

The CytoSelect™ 24-well Wound Healing Assay Kit contains 2 x 24-well plates each containing 12 proprietary treated plastic inserts. The inserts create a wound field with a defined gap of 0.9mm for measuring the migratory and proliferation rates of cells. Migratory cells are able to extend protrusions and ultimately invade and close the wound field. Cell proliferation and migration rates can be determined using manual fixing and microscopic imaging. A fixing solution is provided for stopping cells at specific time points. Cell stain and DAPI stain are also provided for viewing results with light and fluorescence microscopy.

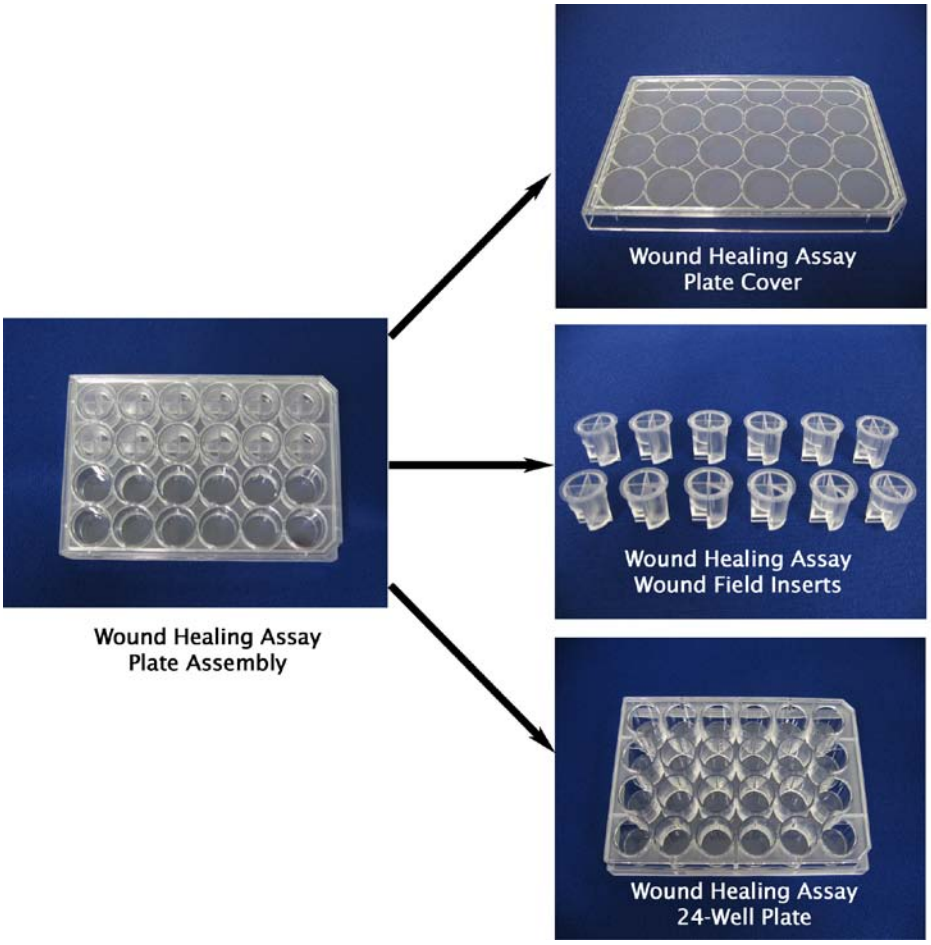


Related Products

1. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8 µm, Colorimetric)
2. CBA-101: CytoSelect™ 24-Well Cell Migration Assay (8 µm, Fluorometric)
3. CBA-102: CytoSelect™ 24-Well Cell Migration Assay (5 µm, Fluorometric)
4. CBA-103: CytoSelect™ 24-Well Cell Migration Assay (3 µm, Fluorometric)
5. CBA-104: CytoSelect™ 96-Well Cell Migration Assay (3 µm, Fluorometric)
6. CBA-105: CytoSelect™ 96-Well Cell Migration Assay (5 µm, Fluorometric)
7. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8 µm, Fluorometric)
8. CBA-107: CytoSelect™ 24-Well Cell Migration Assay (12 µm, Colorimetric)
9. CBA-125: Radius™ 24-Well Cell Migration Assay (Microscopy)
10. CBA-126: Radius™ 96-Well Cell Migration Assay (Microscopy)

Kit Components

1. 24-well Wound Healing Assay Plate (Part No. 112001): Two 24-well plates containing 12 wound field inserts each (see Figure below)
2. Cell Stain Solution (Part No. 11002): One 10 mL bottle
3. DAPI Fluorescence Stain (1000X) (Part No. 112002): One 30 µL vial
4. Fixation Solution (Part No. 122402): One 20 mL bottle



Materials Not Supplied

1. Migratory cell lines and culture medium
2. Light/Fluorescence microscope with DAPI filter (350nm/470nm)
3. Imaging Software for measuring wound closure
4. Forceps
5. PBS

Storage

Upon receipt, transfer the DAPI Fluorescence Stain to -20ºC. Store all other components at 4ºC.

Assay Protocol (Must be under sterile conditions)

I. Cell Migration

1. Allow the 24-well plate with CytoSelect™ Wound Healing Inserts to warm up at room temperature for 10 minutes.

2. Using sterile forceps, orient the desired number of inserts in the plate wells with their "wound field" aligned in the same direction. Ensure that the inserts have firm contact with the bottom of the plate well.
   Note: It is recommended that all samples be tested in triplicate.
3. Create a cell suspension containing 0.5-1.0 x 10^6 cells/ml in media containing 10% fetal bovine serum (FBS).
4. Add 500 µL of cell suspension to each well by carefully inserting the pipet tip through the open end at the top of the insert. For optimal cell dispersion, add 250 µL of cell suspension to either side of the open ends at the top of the insert. Take care to avoid bumping and moving the inserts.
   Note: Adding too much liquid to the well can decrease the quality of the wound field.
5. Incubate cells in a cell culture incubator overnight or until a monolayer forms.
6. **Carefully** remove the insert from the well to begin the wound healing assay. Use sterile forceps to grab and lift the insert slowly from the plate well. Avoid twisting the insert as this will damage the wound field.
7. Slowly aspirate and discard the media from the wells. Wash wells with media to remove dead cells and debris. Finally, add media to wells to keep cells hydrated.
8. Visualize wells under a light microscope. Repeat wash if wells still have debris or unattached cells.
9. When washing is complete, add media with FBS and/or compounds to continue cell culture and wound healing process. Agents that inhibit or stimulate cell migration can be added directly to the wells.
10. Incubate cells in a cell culture incubator.
11. For best results, use a reticle with micrometer measurements to create a defined surface area in order to monitor the closing, or "healing" of the wound. Focus on the center of the wound field. Create the defined surface area by multiplying the width of the wound field (0.9 mm) by the length. See the example in Figure 1 below.

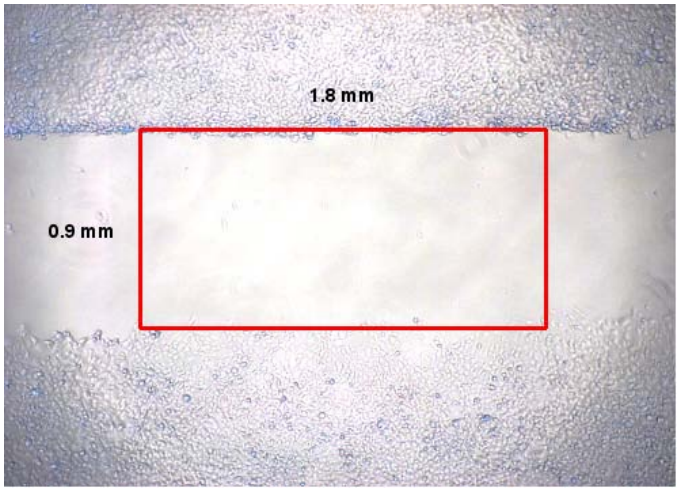


Figure 1: Example of Wound Field Surface Area.

12. Monitor the wound closure with a light microscope or imaging software. Measure the percent closure or the migration rate of the cells into the wound field. Wound healing results can be visualized with phase contrast, DAPI fluorescence labeling, or cell staining.

II. (Optional) DAPI Fluorescence Labeling

1. Cells can be fixed by removing media and adding 0.5 mL of Fixing Solution to each well.
2. Allow the cells to fix for 10 minutes at room temperature. Aspirate and discard the solution.
3. Carefully wash each well 3X with PBS.
4. Dilute DAPI 1:1000 in PBS.
5. Add 0.5 mL of DAPI solution to each well to be stained. Incubate 15 minutes at room temperature.
6. Carefully wash each well 3X with PBS. Add 1mL PBS to each well to keep cells hydrated.

III. (Optional) Cell Staining

1. Remove the media or solution and add 400 µL of Cell Stain Solution to each well.
2. Allow the stain to incubate with the cells for 15 minutes at room temperature. Aspirate and discard the solution.
3. Carefully wash each stained well 3X with deionized water. Discard washes and allow cells to dry at room temperature.

Calculation of Results

Percent Closure:

1. Determine the surface area of the defined wound area (see Figure 1). Total Surface Area = 0.9mm x length
2. Determine the surface area of the migrated cells in to the wound area. Migrated Cell Surface Area = length of cell migration (mm) x 2 x length
3. Percent Closure (%) = Migrated Cell Surface Area / Total Surface Area x 100

Migration Rate:

Determine the migration rate of cells into the defined wound area:

Migration Rate = length of cell migration (nm) / migration time (hr).

Example of Results

The following figure demonstrates typical results with the CytoSelect™ 24-well Wound Healing Assay Kit. This data should not be used to interpret actual results.

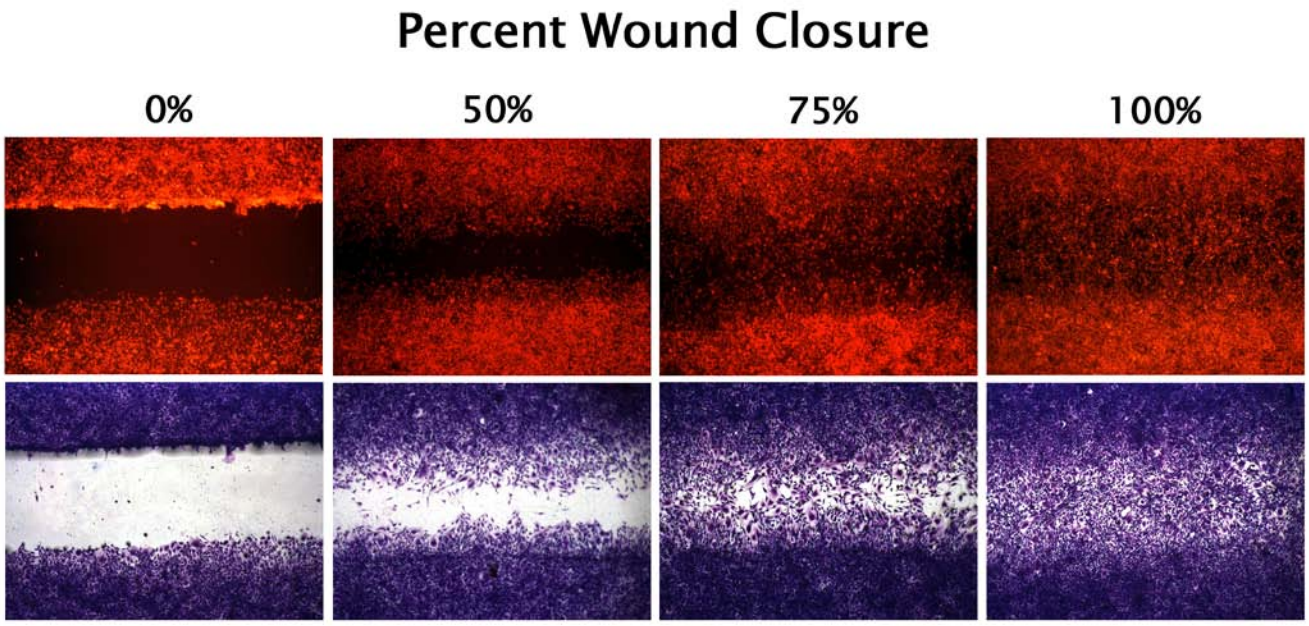


Figure 2: Percent Closure of MEF/STO Cells. STO cells were tested using the CytoSelect™ 24-Well Wound Healing Assay. Cells were cultured 24 hours until a monolayer formed at which time the inserts were removed to begin the wound healing assay. Cells were monitored under phase contrast (not shown), DAPI labeling, and cell staining for determining percent closure (0, 50, 75, and 100%).

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Recent Product Citations

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Please see the complete list of product citations: http://www.cellbiolabs.com/wound-healing-assays.

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