# ABS_Bio™ Scratch Wound Healing Assay Kit

**(Cat# K040-100; 100 assays; store kit at 4℃)**

## Introduction

The scratch wound healing assay has been widely adapted and modified to study the effects of a variety of experimental conditions, for instance, gene knockdown or chemical exposure, on mammalian cell migration and proliferation.

The ABS_Bio™ Scratch Wound Healing Assay Kit provides a simple, sensitive, one-step colorimetric and fluorimetric assay for cell migration and proliferation. In this assay, a "wound gap" in a cell monolayer is created by scratching, and the "healing" of this gap by cell migration and growth towards the center of the gap is monitored and quantitated. Factors that alter the motility and/or growth of the cells can lead to increased or decreased rate of "healing" of the gap. The Cell proliferation and migration rates can be determined using manual fixing and microscopic imaging. The kit is supplied with sufficient reagents for 100 tests in 24-well or 48-well plate assay. It could easily be modified for a high-throughput assay.

## Kit Components (100 tests)

Fixation Solution: 40 mL Light Stain Solution: 40 mL Fluorescence Stain Solution(100x): 0.4 mL

**Storage and Handling: Store Fluorescence Stain Solution at -20℃, others components at 4℃. Shelf Life: 12 months after receipt. Warm up Reagents to room temperature before use.**

## Procedure

1. Grow cells in DMEM supplemented with 10% FBS.
2. Seed cells into 24-well tissue culture plate at a density that after 24 h of growth, they should reach ~80-90% confluence as a monolayer.
3. Do not change the medium. Gently and slowly scratch the monolayer with a new 1 ml pipette tip or mini scraper across the center of the well. While scratching across the surface of the well, the long-axial of the tip should always be perpendicular to the bottom of the well. The resulting gap distance therefore equals to the outer diameter of the end of the tip. The gap distance can be adjusted by using different types of tips. Scratch a straight line in one direction.
4. Scratch another straight line perpendicular to the first line to create a cross in each well.
5. After scratching, gently wash the well twice with medium to remove the detached cells.
6. Replenish the well with fresh medium to start wound healing process.

***Note: Medium may contain ingredients of interest that you want to test, e.g., chemicals that inhibit/promote cell motility and/or proliferation.***

7. Grow cells for additional 24-48h (or the time required if different cells are used). Monitor the wound closure with a light microscope or image assay (see below cell stain).
8. Measure the percent closure or the migration rate of the cells into the wound field.

## Calculation of Results

**Percent Closure:**

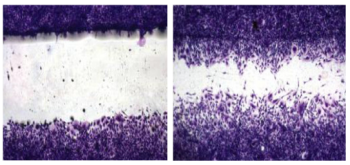
1. Determine the surface area of the defined wound area. Total Surface Area = wide x length (mm)
2. Determine the surface area of the migrated cells in to the wound area. Migrated Cell Surface Area = wide of cell migration (mm) x 2 x length (mm)
3. Percent Closure (%) = Migrated Cell Surface Area / Total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

**Cell stain Assay**

1. Wash the cells twice with 1x PBS.
2. Fix the cells with 400 µL fixation solution for 15 min at RT
3. Aspirate and discard the solution. Wash the cells twice with 1x PBS.



Cells light stain assay

**For light stain**: Stain the fixed cells with 400 µL Light Stain Solution for 15 min at RT, wash the cells twice with 1x PBS, discard washes and allow cells to dry at room temperature. Shoot pictures with light microscope.

**For fluorescence stain**: 100x dilute Fluorescence Stain Solution with PBS, add 400 µL diluted stain solution into each well for 15 min at RT, wash the cells twice with 1x PBS. Shoot pictures with Fluorescence microscope with 350nm/470nm filter.

The gap distance can be quantitatively evaluated using software such as Photoshop or ImageJ (http://rsb.info.nih.gov/ij/download.html)

## References

Liang, CC. et al. 2007, Nature Protocol 2:329-333
Yarrow, JC. et al. 2004, BMC Biotechnology 4:21

## Related Products:

24-Well plate with 24 ready to use Culture-Inserts for wound healing assay (#S80241)

Culture-Insert for wound healing assay (#S80209)