![Axion Biosystems logo]

Scratch Assay Protocol

Imaging systems – Collective cell migration of adherent cells

Workflow

Seed cells at high density	Create scratch	Image scratch closure at regular time intervals
Day -1	**Day 0**	**Day 0+**

This protocol details the use of live-cell imaging to monitor collective cell migration with a scratch assay. The **Omni** or **Lux3** can be used to image scratch closure over time. Once the images are uploaded in the Axion Portal, the integrated **Scratch Assay Module** analyzes the scratch area at each time point. This protocol describes a manual scratch assay, however automated scratching devices or cell culture inserts can also be used to create a "scratch".

Cell culture

1. Thaw and culture cells of interest in accordance with supplier recommendations and passage as needed.

2. Detach and count the cells in order to dilute the cell suspension to the desired working concentration.

Seeding cells

8. Add the appropriate amount of cell suspension to each well such that the monolayer reaches 75-90% confluency by the start of the experiment (Fig. 1). Make sure to homogeneously spread the cells during seeding.

9. Place the cells in the incubator for at least 24 hours to form a cell monolayer. Avoid shaking the culture vessel as this will result in an inhomogeneous cell distribution.

10. If desired, add a step to limit cell proliferation to ensure scratch closure is solely caused by migration. This can be done by overnight serum starvation or addition of mitomycin C, a DNA synthesis inhibitor.

Tip

To find the correct seeding density, perform a test with different seeding densities such that the appropriate confluency (75 – 90%) is reached after 24 h (Figure 1).
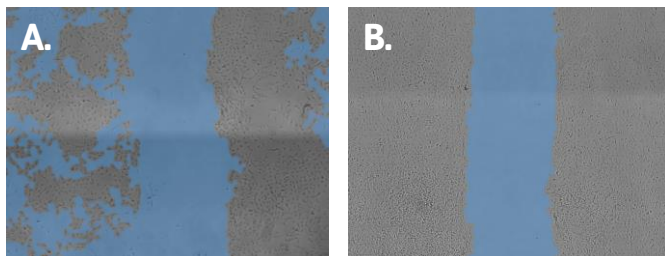

Figure 1: The effect of confluency on scratch analysis
Overlay of the detected scratch area (blue) of C6 cells with 55% (A) and 76% confluency (B). In case the confluency is too low, gaps in the scratch edge cause "bleeding" of the scratch area.

Performing the scratch assay

11. Check the cell density after 24 h with the **Omni** or **Lux3**. In case the confluency is lower than 75%, place the cells back in the incubator and check the confluence regularly until the correct level is reached (Fig. 1).

12. Prewarm culture medium and PBS. If applicable, add test compounds to the medium.

13. Create scratches using a p200 pipette tip. Place the pipette tip next to the wall of the well such that it is exactly perpendicular to the well bottom. Drag the tip to the other side of the well while applying gentle pressure.

14. Aspirate the medium. Make sure not to touch the scratch while aspirating.

15. Add PBS and swirl gently to release all scratched cells from the bottom. Aspirate the PBS. Repeat this process in case detached cells are still visible in the scratch area.

16. Add cell culture medium (with test compounds).

Live-cell imaging

17. Place the well plate on the **Omni** or **Lux3** located in a cell culture incubator.

18. Select the full scan mode or brightfield channel. Subsequently focus such that there is a clear contrast between the scratch and the cells.

19. Select the **Scratch Assay Module** and a time-lapse experiment. Choose a 30 minute or 1-hour interval for 24-hour experiments or a 2-hour interval for 48-hour experiments.

Analysis of scratch closure

20. Go to the Axion Portal and open the experiment.

21. Toggle on the **Scratch Assay Module** to get an overlay of the scratch area on top of the brightfield image (Fig. 2).

22. Scratch closure percentage (Fig. 3B) can be calculated from scratch area (Fig. 3A) using the following formula:

$$Scratch\ closure = \frac{A_0 - A_t}{A_0} \cdot 100\%$$

A_0: scratch area at the first timepoint, A_t: scratch area at the current timepoint.

> **Tip**
> **1:** Try different amounts of pressure before performing the actual experiment. If the pressure is too low, not all cells will be removed. If the pressure is too high, it might damage the plate surface.
>
> **2:** Use a ruler or the lid of the plate to guide the pipette tip to create a straight scratch.

> **Tip**
> Wash with culture medium instead of PBS in case delicate cells are used.

> **Tip**
> Drawing a smaller analysis area around the scratch might improve the scratch analysis in case holes in the monolayer are found outside the scratch area. This is only possible with **Omni** experiments.
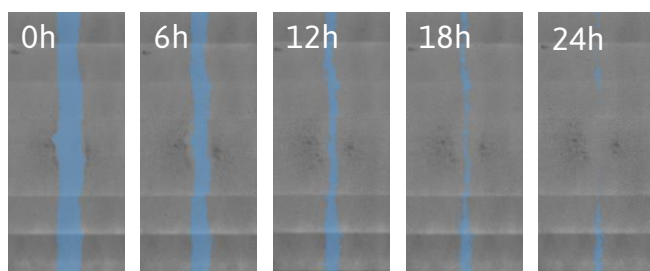
Figure 2: Example scratch analysis.
Typical example of the decrease in scratch area over time. The blue overlay indicates the detected scratch area after 0, 6, 12, 18 and 24 hours of C6 cell migration.
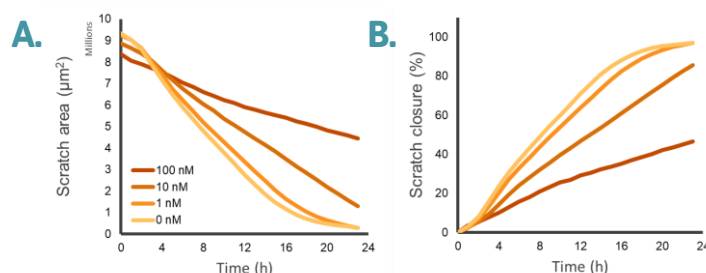
Figure 3: Example scratch assay experiment.
Scratch area (A) and percentage of scratch closure (B) over time of C6 cells treated with 0, 1, 10 and 100 mM of paclitaxel. The time interval was 1 hour and the average of 6 samples per group is shown.

Required Materials

Consumables

Item	Vendor
Well-plate (6 – 96 wells)	Various
Culture medium	Various
PBS	Various
200 µl pipette tips	Various

Equipment

Item	Vendor
Omni (FL) or Lux3 (FL)	Axion BioSystems
Hemocytometer or cell counter (e.g. Exact FL)	Various
Pipet controller	Various