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Scratch-wound assay

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ABSTRACT

This protocol describes the process for setting up a scratch-wound assay analysed by the IncuCyte system.

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Seed cells at a density of 20,000 cells/well (might need optimisation for a particular cell line) in ImageLock 96-well plates. I usually plate replicates in columns as that way you can easily calculate the mean and export from the IncuCyte software directly.

2 Incubate overnight (12-16 hours) or until confluent (make sure you always keep the same incubation time for consistency)

3 Create the wound using the WoundMaker:
Remove top of WoundMaker and set top in empty wash boat.
Rinse the Wound Maker in 70% ethanol, PBS and then use for making the wound. Rinse with ethanol after use. Never flip it over.
Insert plate (containing cells & media) into base plate holder. Remove plate cover.
Replace pin block by guiding the rear dowels of pin block into the rear holes of the base plate. Do not push down.
Push and hold the black lever.
Lift pin block while continuing to hold the black lever down.
Replace plate cover. Wash wells (up to two washes).

4 Immediately aspirate the media from each well and carefully wash the cells twice with culture media (100 µL/well). Be gentle while adding the medium and aspirating as the confluent layer of cells might easily detach).

5 After washing, add 100 µl of culture media

6 Place the cell plate into the IncuCyte live-cell analysis system and allow the plate to warm to 37°C for 30 minutes prior to scanning.
a. Objective: 4x, 10x (recommended), or 20x
b. Channel selection: Phase Contrast (+ Fluorescence if analyzing cells with fluorescent labels)
c. Scan type: Scratch Wound (Wide Mode optional for 10x, required for 20x)

7 Incubate overnight and check if wound is closed.

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