Protocol

IncuCyte® Label-Free Cell Proliferation Assay

For counting and confluence measurements of adherent or non-adherent cell lines

This protocol provides an overview of the IncuCyte® Cell Proliferation Assay methodology. It is compatible with the IncuCyte® Live-Cell Analysis System for kinetic, label-free analysis of cell confluence or cell counts using your choice of cells and treatments.

The highly flexible assay format can be combined with our range of IncuCyte® cell health and viability reagents for multiplexed measurements of cytotoxicity and apoptosis alongside proliferation in the same well.

Required materials

- Flat bottom tissue culture plate (e.g., Corning Cat. No. 3595)

Optional materials

- IncuCyte® Cell-By-Cell Analysis Software Module (Sartorius Cat. No. 9600-0031)—for label-free cell counting
- Poly-L-ornithine (Sigma Cat. No. P4957)—for non-adherent cells
- Fibronectin (Sigma Cat. No. A7906)—for non-adherent cells

General guidelines

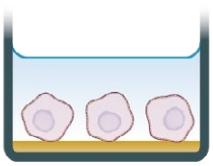
- Following cell seeding, place plates at ambient temperature (15 minutes for adherent cell lines and 45 minutes for non-adherent cell lines) to ensure homogenous cell settling.
- Remove bubbles from all wells by gently squeezing a wash bottle containing 70-100% ethanol with the inner straw removed, to blow vapor over the surface of each well.
- After placing the plate in the IncuCyte Live-Cell Analysis System, allow the plate to warm to 37° C for 30 minutes prior to scanning.

Quick guide

1. Coat wells

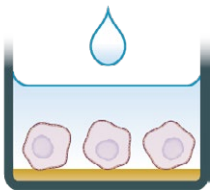
Coat wells of plate (50 µL/well) with appropriate matrix. Optional for adherent cell lines.

2. Plate cells



Seed cells (100 µL/well, 1,000–10,000 for adherent and 5,000–50,000 for non-adherent) into a 96-well plate.

3. Add treatments



Add desired treatments (100 µL/well, 1x for adherent cell lines, 2x for non-adherent cell lines).

Adherent cell line protocol

Day 0

1. Coat wells (optional)

1.1 Depending on cell line used, coat a 96-well flat bottom plate with relevant coating matrix according to manufacturer's recommendation.

1.2 Prior to cell seeding, prepare cell treatments at 2x final assay concentration in enough cell culture medium to achieve a volume of 100 µL per well.

2. Plate cells

2.1 Seed your choice of cells (100 µL per well) at an appropriate density into a 96-well plate, such that by day 1 the cell confluence is approximately 10–20%. The seeding density will need to be optimized for the cell line used; however, we have found that 1,000 to 2,500 cells per well (10,000 to 25,000 cells/mL seeding stock) are reasonable starting points.

a. Monitor cell growth using the IncuCyte® Live-Cell Analysis System to capture phase contrast images every two hours and analyze using the integrated confluence algorithm.

Day 1

3. Add treatments

3.1 Prepare 1x concentrations of desired cell treatments in cell culture medium. The volumes may be varied; however, we recommend preparing enough volume of each desired treatment/dilution in order to achieve 100 µL per well.

3.2 Remove the cell plate from the incubator and aspirate medium from wells.

3.3 Add treatments and controls to appropriate wells of the 96-well plate.

3.4 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 or 20x
b. Channel selection: Phase Contrast (+ "Green" or "Red" if fluorescent label or cell health reagents are used)
c. Scan type: Standard
d. Scan interval: Typically, every 1 to 2 hours, until your experiment is complete.

NOTE: Label free cell counting can be enabled on IncuCyte Live-Cell Analysis System with use of the IncuCyte Cell-by-Cell Analysis Software Module.

a. Scan type: Standard/Adherent Cell-by-Cell
b. Objective: 10x

For further details of this analysis module and its application see: **www.essenbioscience.com/cell-by-cell**

Non-adherent cell line protocol

Day 1

1. Seed cells and add prepared treatments

1.1 Coat a 96-well flat bottom plate with relevant coating matrix. We recommend coating with 50 µL of either 0.01% poly-L-ornithine solution or 5 µg/mL fibronectin diluted in 0.1% BSA. Coat plates for 1 hour at ambient temperature, remove solution from wells, then allow plates to dry for 30-60 minutes prior to cell addition.

1.2 Prior to cell seeding, prepare cell treatments at 2x final assay concentration in enough cell culture medium to achieve a volume of 100 µL per well.

2. Plate cells

2.1 Seed your choice of cells (100 µL per well) at an appropriate density into a 96-well plate. The seeding density will need to be optimized for the cell line used; however, we have found that 5,000 to 50,000 cells per well (50,000 – 500,000 cells/mL seeding stock) are reasonable starting points.

NOTE: If studying immune cell clustering and proliferation, prepare cell activation treatments at 5x final concentration, and immediately add 50 µL per well containing cells. It is advised that some control wells containing only vehicle are included in the plate.

3. Add treatments

3.1 Immediately after cell seeding, add treatments and controls to appropriate wells of the 96-well plate containing cells. Triturate wells to appropriately mix the treatment to ensure cell exposure at 1x.

3.2 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 (recommended 1 image per well or whole well) or 10x
b. Channel selection: Phase Contrast (+ "Green" or "Red" if fluorescent label or cell health reagents are used)
c. Scan type: Standard
d. Scan interval: Typically, every 1 to 2 hours, until your experiment is complete.

NOTE: Label-free cell counting can be enabled on IncuCyte Live-Cell Analysis System with use of the IncuCyte Cell-by-Cell Analysis Software Module.

a. Scan type: Standard/Non-Adherent Cell-by-Cell
b. Objective: 20x

For further details of this analysis module and its application see: **www.essenbioscience.com/cell-by-cell**

Related products and applications:

A comprehensive range of fluorescent nuclear labeling and cell health reagents are available for use with the IncuCyte Live-Cell Analysis System to enable multiplexed measurements of cytotoxicty and proliferation alongside apoptosis.

Compatible with the IncuCyte® Live-Cell Analysis System Cat. No. 4647

Product	Cat. No.	Amount
IncuCyte® NucLight Green Lentivirus Reagent (EF-1α, Puro) for nuclear labeling	4624	0.2 mL
IncuCyte® NucLight Red Lentivirus Reagent (EF-1α, Puro) for nuclear labeling	4625	0.2 mL
IncuCyte® NucLight Green Lentivirus Reagent (EF-1α, Bleo) for nuclear labeling	4626	0.2 mL
IncuCyte® NucLight Red Lentivirus Reagent (EF-1α, Bleo) for nuclear labeling	4627	0.2 mL
IncuCyte® NucLight Green Lentivirus Reagent (EF-1α, Puro) for nuclear labeling	4475	0.6 mL
IncuCyte® NucLight Red Lentivirus Reagent (EF-1α, Puro) for nuclear labeling	4476	0.6 mL
IncuCyte® NucLight Green Lentivirus Reagent (EF-1α, Bleo) for nuclear labeling	4477	0.6 mL
IncuCyte® NucLight Red Lentivirus Reagent (EF-1α, Bleo) for nuclear labeling	4478	0.6 mL
IncuCyte® Cytotox Red Reagent for counting dead cells	4632	5 µL x 5
IncuCyte® Cytotox Green Reagent for counting dead cells	4633	5 µL x 5
IncuCyte® Annexin V Red Reagent for apoptosis	4641	100 tests
IncuCyte® Annexin V Green Reagent for apoptosis	4642	100 tests
IncuCyte® Caspase-3/7 Red Reagent for apoptosis	4704	20 uL
IncuCyte® Caspase-3/7 Green Reagent for apoptosis	4440	20 µL

Compatible with the IncuCyte® Live-Cell Analysis System for Neuroscience Cat. No. 4763

Product	Cat. No.	Amount
IncuCyte® NucLight Orange Lentivirus Reagent (EF-1α, Puro) for nuclear labeling	4771	0.2 mL
IncuCyte® Annexin V Orange Reagent for apoptosis	4760	100 tests
IncuCyte® Annexin V NIR Reagent for apoptosis	4768	100 tests

A complete suite of cell health applications is available to fit your experimental needs. Find more information at **www.sartorius.com/incucyte**

For additional product or technical information, please email us at **AskAScientist@sartorius.com**

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