

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® Label-Free 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.

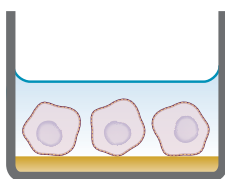
Quick Guide

1. Coat wells



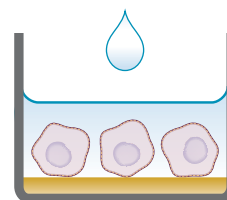
Coat wells of plate (50 μ L/well) with appropriate matrix. Optional for adherent cell lines.

2. Plate cells



Seed cells (100 μ L/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 μ L/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 μ L per well.

2. Plate Cells

- 2.1 Seed your choice of cells (100 μ L 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.

- 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 (+ Fluorescence 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

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 μ L per well.

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 μ L of either 0.01% poly-L-ornithine solution or 5 μ g/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 μ L per well.

2. Plate Cells

- 2.1 Seed your choice of cells (100 μ L 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 5,000–50,000 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 μ L 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 cytotoxicity and proliferation alongside apoptosis.

Compatible with the Incucyte® Live-Cell Analysis System

Proliferation & Cell Cycle				
Product	Color	Quantity	Compatibility	Cat. No.
Incucyte® Nuclight Green Lentivirus (puro)		One vial: 0.2 mL	SX1, S3, SX5	4624
		One vial: 0.6 mL		4475
Incucyte® Nuclight Green Lentivirus (bleo)		One vial: 0.2 mL	SX1, S3, SX5	4626
		One vial: 0.6 mL		4477
Incucyte® Nuclight Red Lentivirus (puro)		One vial: 0.2 mL	SX1, S3, SX5 (Green Red Optical Module)	4625
		One vial: 0.6 mL		4476
Incucyte® Nuclight Red Lentivirus (bleo)		One vial: 0.2 mL	SX1, S3, SX5 (Green Red Optical Module)	4627
		One vial: 0.6 mL		4478
Incucyte® Nuclight Orange Lentivirus (puro)		One vial: 0.2 mL	S3 for Neuroscience, SX5	4771
Incucyte® Nuclight NIR Lentivirus (puro)		One vial: 0.2 mL	S3 for Neuroscience, SX5	4805
Incucyte® Nuclight Rapid Red Dye		One vial: 50 μ L	SX1, S3, SX5 (Green Red Optical Module)	4717
Incucyte® Nuclight Rapid NIR Dye		One vial: 50 μ L	S3 for Neuroscience, SX5	4804
Incucyte® Cell Cycle Green Red Lentivirus (puro)		One vial: 0.6 mL	SX1, S3, SX5 (Green Red Optical Module)	4779
Incucyte® Cell Cycle Green Orange Lentivirus (puro)		One vial: 0.6 mL	SX5	4809

Apoptosis & Cytotoxicity				
Product	Color	Quantity	Compatibility	Cat. No.
Incucyte® Annexin V Green Dye		One vial: 100 tests	SX1, S3, SX5	4642
Incucyte® Annexin V Red Dye		One vial: 100 tests	SX1, S3, SX5 (Green Red Optical Module)	4641
Incucyte® Annexin V NIR Dye		One vial: 100 tests	S3 for Neuroscience, SX5	4768
Incucyte® Annexin V Orange Dye		One vial: 100 tests	S3 for Neuroscience, SX5	4759
Incucyte® Caspase-3/7 Green Dye		One vial: 20 µL	SX1, S3, SX5	4440
Incucyte® Caspase-3/7 Red Dye		One vial: 20 µL	SX1, S3, SX5 (Green Red Optical Module)	4704
Incucyte® Cytotox Green Dye		Five vials: 5 µL each	SX1, S3, SX5	4632
Incucyte® Cytotox Red Dye		Five vials: 5 µL each	SX1, S3, SX5 (Green Red Optical Module)	4633

*Pre-labeled Nuclight cell lines are also available for purchase. Please visit shop.essenbioscience.com for more information

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

For Research Use Only. Not For Therapeutic or Diagnostic Use.

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