



# IncuCyte® Cell Proliferation Assay

For label-free proliferation 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 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 3595)
- Poly-L-ornithine (Sigma P4957) – optional, for non-adherent cells
- Fibronectin (Sigma A7906) – optional, 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.

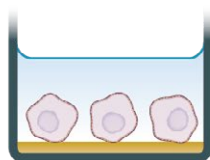
## Quick Guide

### 1 COAT WELLS



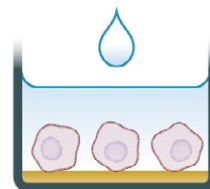
Coat wells of plate (50 µL/well) with appropriate matrix. Optional for adherent cell lines.

### 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  $\mu$ L per well.

#### Plate Cells

- 2.1 Seed your choice of cells (100  $\mu$ 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® system to capture phase contrast images every 2 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  $\mu$ L 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.

## Non-Adherent Cell Line Protocol

### For label-free proliferation measurements of adherent or non-adherent cell lines

### 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  $\mu$ L of either 0.01% poly-L-ornithine solution or 5  $\mu$ 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  $\mu$ L per well.

#### Plate Cells

- 2.1 Seed your choice of cells (100  $\mu$ 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 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  $\mu$ 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



## 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

Product	Cat No.	Amount
IncuCyte® NuLight Green BacMam 3.0 Reagent for nuclear labeling	4622	1 mL
IncuCyte® NuLight Green Lentivirus Reagent (EF-1 $\alpha$ , Puro) for nuclear labeling	4624	0.2 mL
IncuCyte® NuLight Red Lentivirus Reagent (EF-1 $\alpha$ , Puro) for nuclear labeling	4625	0.2 mL
IncuCyte® NuLight Green Lentivirus Reagent (EF-1 $\alpha$ , Bleo) for nuclear labeling	4626	0.2 mL
IncuCyte® NuLight Red Lentivirus Reagent (EF-1 $\alpha$ , Bleo) for nuclear labeling	4627	0.2 mL
IncuCyte® NuLight Green Lentivirus Reagent (EF- $\alpha$ , Puro) for nuclear labeling	4475	0.6 mL
IncuCyte® NuLight Red Lentivirus Reagent (EF-1 $\alpha$ , Puro) for nuclear labeling	4476	0.6 mL
IncuCyte® NuLight Green Lentivirus Reagent (EF-1 $\alpha$ Bleo) for nuclear labeling	4477	0.6 mL
IncuCyte® NuLight Red Lentivirus Reagent (EF-1 $\alpha$ , Bleo) for nuclear labeling	4478	0.6 mL
IncuCyte® Cytotox Red Reagent for counting dead cells	4632	5 $\mu$ L x 5
IncuCyte® Cytotox Green Reagent for counting dead cells	4633	5 $\mu$ L 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 Green Reagent for apoptosis	4440	20 $\mu$ L

A complete suite of cell health applications is available to fit your experimental needs. Find more information at [essenbioscience.com](http://essenbioscience.com)

For additional product or technical information, please e-mail us at [AskAScientist@essenbio.com](mailto:AskAScientist@essenbio.com) visit our website at [essenbioscience.com](http://essenbioscience.com)

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