

IncuCyte™ Cell Count Proliferation Assay General Protocol

This protocol provides an overview of the IncuCyte™ Cell Count Proliferation Assay methodology. It is compatible with the IncuCyte ZOOM® live-cell imaging system and enables real-time cell counting using your choice of cells and treatments. The IncuCyte™ NuLight™ range of live cell labelling reagents are used to fluorescently label the nuclei of living cells without perturbing cell function or biology.

Transient or stable expression strategies are supported using either the IncuCyte™ NuLight™ BacMam 3.0 or NuLight™ Lentivirus range of reagents. In addition, the highly flexible assay format can be combined with our range of IncuCyte™ Cytotox Reagents or the IncuCyte™ Caspase 3/7 reagent for multiplexed measurements of cytotoxicity and apoptosis alongside proliferation in the same well.

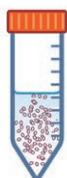
Nuclear labeling by transient transduction

We recommend the use of IncuCyte™ NuLight™ BacMam 3.0 Reagents to transiently transduce your choice of cells. As well as eliminating the need to create stable cell lines these reagents enable the rapid expression of a nuclear-restricted green or red

fluorescent protein (GFP or mKate2) in your choice of primary, immortalized, dividing or non-dividing mammalian cells without altering cell function and with minimal toxicity.

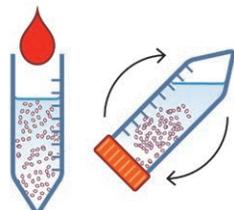
Quick guide

1 HARVEST CELLS



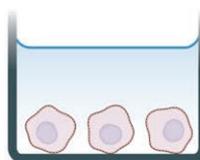
Prepare Cell Seeding Stock
Harvest cells and resuspend at 2×10^4 cells/mL in full growth medium.

2 TRANSDUCE



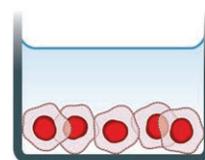
Add IncuCyte™ NuLight™ BacMam 3.0 Reagent
Add Green or Red NuLight™ BacMam 3.0 Reagent (1 to 2% (v/v)). Mix by inversion.

3 SEED CELLS



Seed Cells
Seed cells (100µL/well, 2×10^3 cells/well) and incubate at ambient temperature for 30 minutes.

4 LIVE CELL FLUORESCENT IMAGING



Automated Imaging and Quantitative Analysis
Capture images every 1 to 2 hours (4x, 10x or 20x) in an IncuCyte ZOOM® system. Analyze using integrated software.

General Protocol

Day 0

1. Harvest cells and dilute to 20,000 cells/mL in a conical tube.
- 2) Note: Cultures should be below 80% confluence at time of harvest, and at least 90% viable, prior to transduction. Overly confluent or unhealthy cells will result in inefficient transduction.
- 3) Add IncuCyte™ NuLight™ BacMam 3.0 reagent to cells and mix by inversion. The concentration of IncuCyte™ NuLight™ BacMam 3.0 reagent used will need to be optimized for each cell type used; however we have found that concentrations of 0.5 to 4% (v/v) are reasonable starting points. We recommend running our optimization protocol prior to setting up proliferation assays.
- 4) Seed the transduced cells (100 µL per well, 2,000 cells per well) into a 96-well flat bottom plate and allow the plate to incubate at ambient temperature for 20 minutes prior to scanning

- 5) Place the plate into the IncuCyte ZOOM® 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 + Fluorescence
 - c. Scan type: Standard
 - d. Scan interval: Typically every 2 hours

Day 1

1. Once the infected cells have started to fluoresce (usually after 18 - 21 hours), remove the cell plate from the incubator and add desired treatments. The volumes/dilutions may be varied; however, we recommended aspirating the culture medium and adding 100 µL of the desired treatment, prepared at 1x final assay concentration. Continue to image the plate in the IncuCyte ZOOM® system until your experiment is complete.

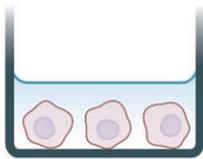
Creating a cell population or clone that stably expresses a nuclear label

We recommend the use of IncuCyte™ NuLight™ Lentivirus Reagents to provide stable, homogenous expression of a nuclear-restricted green or red fluorescent protein (GFP or mKate2) in your choice of living mammalian cells without

perturbing cell function and with minimal toxicity. These reagents are ideal for generating stable cell populations or clones using puromycin or bleomycin selection.

Quick guide

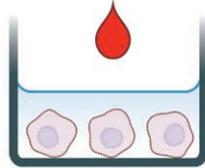
1 SEED CELLS



Cell Seeding

Seed cells in growth media and leave to adhere (4-24 hours). Cells should be 15-35% confluent at the time of transduction.

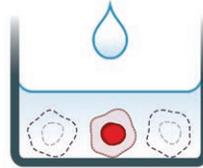
2 TRANSDUCE



Add IncuCyte™ NuLight™ Lentivirus Reagent

Add Green or Red NuLight™ Lentivirus Reagent (MOI 3 to 6) diluted in media ± Polybrene®. After 24 hours, replace the media with fresh growth media. Monitor expression using the IncuCyte ZOOM® system.

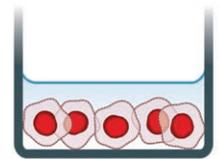
3 APPLY SELECTION



Generate a Stable Population or Clone

Apply antibiotic selection to derive a stable, homogenous cell population or clone that expresses a nuclear restricted green or red fluorescent protein. (Optional: Freeze cells and use for future assays).

4 LIVE CELL FLUORESCENT IMAGING



Automated Imaging and Quantitative Analysis

Capture images every 1 to 2 hours (4x, 10x or 20x) in an IncuCyte ZOOM® system. Analyze using integrated software.

General Protocol

1. Seed cells in growth media of choice and at a density such that they are 15-35% confluent after 24 hours of incubation.
2. Add the IncuCyte™ NuLight™ Lentivirus Reagent at desired multiplicity of infection (MOI = TU/cell). An MOI of 3 to 6 is recommended for most cell types, however, an optimized MOI should be determined for each cell type used. Polybrene® (1-8 µg/mL) may also be added to enhance transduction of some cell types (Note: Certain cell types can be sensitive to Polybrene® (e.g. neurons)).
3. Incubate at 37°C, 5% CO₂ for 24 hours, then remove and replace with fresh growth media.
4. Return to incubator for an additional 24-48 hours, monitoring expression using an IncuCyte ZOOM® system.
5. Remove media and replace with fresh growth medium containing selection (i.e. puromycin or bleomycin). Example: For HT-1080, A549, HeLa, and MDA-MB-231 cells, complete media containing 1 µg/mL puromycin is sufficient for efficient killing of non-transduced cells.
6. Incubate for 72-96 hours, replacing media every 48 hours. Maintain stable population in a maintenance concentration of selection media. Example: HT-1080, A549, HeLa, and MDA-MB-231 cells labelled with the IncuCyte™ NuLight™ Red Lentivirus Reagent (EF-1 µ, Puro) can be maintained in complete media containing 0.5 µg/mL puromycin.