Protocol

IncuCyte® Cell Count Proliferation Assay

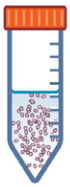
This protocol provides an overview of the IncuCyte® Cell Count Proliferation Assay methodology. It is compatible with the IncuCyte® S3 Live-Cell Analysis System and enables real-time cell counting using your choice of cells and treatments. The IncuCyte® NucLight range of live-cell labelling reagents 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® NucLight BacMam 3.0 or NucLight 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® NucLight Green BacMam 3.0 Reagent to transiently transduce your choice of cells. As well as eliminating the need to create stable cell lines. This reagent enables the rapid expression of a nuclear-restricted green fluorescent protein (GFP) in your choice of primary, immortalized, dividing or non-dividing mammalian cells without altering cell function and with minimal toxicity.

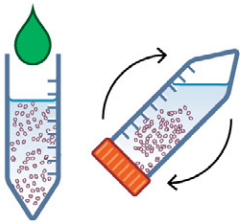
Quick Guide

1. Harvest cells



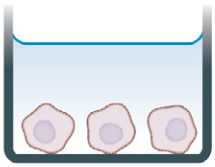
Prepare Cell Seeding Stock
Harvest cells and resuspend at 2 x 10^4 cells/mL in full growth medium.

2. Transduce



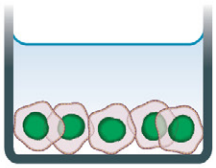
Add Incucyte® Nuclight Green BacMam 3.0 Reagent
Add NucLight BacMam 3.0 Reagent (1 to 2% (v/v)). Mix by inversion.

3. Seed cells



Seed Cells
Seed cells (100µL/well, 2 x 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® S3 Live-Cell Analysis System. Analyze using integrated software.

General Protocol

Day 0

1. Harvest cells and dilute to 20,000 cells/mL in a conical tube.

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.

2. Add IncuCyte® NucLight BacMam 3.0 reagent to cells and mix by inversion.

The concentration of IncuCyte NucLight 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.

3. 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.

4. Place the plate into the IncuCyte® S3 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 + Fluorescence
c. Scan type: Standard
d. Scan interval: Typically every 2 hours

NOTE: Cell counting can be enabled on IncuCyte S3 Live-Cell Analysis System with use of the IncuCyte Cell-by-Cell Analysis Software Module (PN 9600 0031). This enables individual cell identification and subsequent classification into subpopulations based on properties including fluorescence intensity, size and shape.

e. Scan type: Standard/Adherent Cell-by-Cell
f. Objective: 10x

For further details of this analysis module and it's application see:
www.essenbioscience.com/cell-by-cell

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.

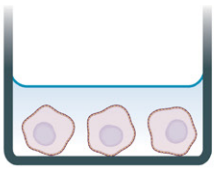
The volumes/dilutions may be varied; however, we recommended aspirating the culture medium and adding 100 µL of the desired treatment, prepared at 1x final assay concentration. Continue to image the plate in the IncuCyte® S3 Live-Cell Analysis System until your experiment is complete.

Creating a cell population that stably expresses a nuclear label

We recommend the use of IncuCyte® NucLight 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.

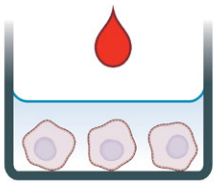
Quick Guide

1. Seed cells



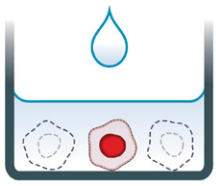
Cell Seeding
Seed cells in growth media and leave to adhere (4-24 hours). Cells should be 15-35% confluent at the time of transduction.

2. Transduce



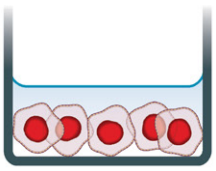
Add Incucyte® Nuclight Lentivirus Reagent
Add Green or Red NucLight Lentivirus Reagent (MOI 3 to 6) diluted in media ± Polybrene®. After 24 hours, replace the media with fresh growth media. Monitor expression using the IncuCyte® S3 Live-Cell Analysis System.

3. Apply selection



Generate a Stable Population or Clone
Apply anitobiotic 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® S3 Live-Cell Analysis System. Analyze using integrated software.

General Protocol

Day 0

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 NucLight 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® S3 Live-Cell Analysis 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 labeled with the IncuCyte NucLight Red Lentivirus Reagent (EF-1 α, Puro) can be maintained in complete media containing 0.5 µg/mL puromycin.

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

For additional product or technical information,
please e-mail us at **AskAScientist@sartorius.com**
visit our website at **essenbioscience.com**
or call
+1-734-769-1600 (USA)
+44-1707-358688 (Europe)
+81-3-5579-6200 (Japan)