IncuCyte® NucLight BacMam 3.0 Reagents for Nuclear Labeling

Transiently transduce cells with a nuclear restricted fluorescent label.

Presentation, storage and stability

IncuCyte® NucLight™ BacMam 3.0 reagents are supplied as 1 mL vials of virus at a concentration of approximately 1 x 10^8 viral particles per mL sufficient for transducing five to ten 96-well microtiter plates at 1 to 2% (v/v). The IncuCyte® NucLight™ BacMam 3.0 reagent should be stored at +4°C and away from light. Do not freeze. When stored as described, the IncuCyte® NucLight™ BacMam 3.0 reagent will be stable for at least 6 months from the date of receipt.

Background and intended use

IncuCyte® NucLight™ BacMam 3.0 Reagents enable efficient, non-perturbing, nuclear labeling of living mammalian cells. They are compatible with simple mix-and-read protocols, and enable real-time cell counting and the calculation of cell doubling times. IncuCyte® NucLight™ BacMam 3.0 Reagents enable the rapid expression of a nuclear-restricted GFP (green fluorescent protein) or mKate2 (red fluorescent protein) in your choice of primary, immortalized, dividing or non-dividing mammalian cells without altering cell function and with minimal toxicity. The IncuCyte® NucLight™ BacMam 3.0 Reagents can be easily used to transiently transduce your choice of cells eliminating the need to create stable cell lines. The NucLight™ BacMam 3.0 Reagents have been validated for use with the IncuCyte® S3 live cell imaging platform and enable real-time cell counting. Furthermore, the IncuCyte® NucLight™ BacMam 3.0 Reagents can be combined with the IncuCyte® S3 our range of IncuCyte® Cytotox Reagents or the IncuCyte® Caspase 3/7 reagent for multiplexed measurements of cytotoxicity and apoptosis alongside proliferation in a single well.

Recommended use

The IncuCyte® NucLight™ BacMam 3.0 Reagent should be stored at +4°C and away from light (do not freeze). We recommend that the IncuCyte® NucLight™ BacMam 3.0 Reagent is prepared in full media at a concentration of 1 to 2% (v/v) depending on the number of cells being seeded and the cell type being infected. For efficient transduction cells can be resuspended in media containing the IncuCyte® NucLight™ BacMam 3.0 Reagent prior to seeding. Alternatively if cells have already been plated, a full media exchange may be performed with media containing the IncuCyte® NucLight™ BacMam 3.0 Reagent to infect cells. When used in an IncuCyte® S3 live cell imaging system, we recommend data collection every 2 hours for proliferation assays. Please see the relevant protocol published on our website: essenbioscience.com/nuclight

Figure 1. Representative images of primary (HUVEC) and tumor (HT-1080) cell types transduced using the IncuCyte® NucLight™ BacMam Green or Red 3.0 Reagents. Note the nuclear restricted expression of red fluorescent protein (mKate2) or green fluorescent protein (GFP) and the healthy cell morphology.
Safety data sheet (SDS) information

The SDS can be found on our website: essenbioscience.com/nuclight

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

3. SEED CELLS
   - Seed cells (100μL/well, 2 x 10^3 cells/well) and incubate at ambient temperature for 30 minutes.

4. LIVE CELL FLOURESCENT 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.

Figure 2. Concentration-dependent inhibition of proliferation by cycloheximide in HT-1080 fibrosarcoma cells labeled with the IncuCyte® Nuclight™ Green BacMam 3.0 Reagent.

(A) Time-course of nuclear count in the absence (open symbols) and increasing concentrations of cycloheximide (progressively darker blue symbols).

(B) Concentration response curve to cycloheximide. Area under the curve (AUC) values have been determined from the time-course shown in panel A and compared to uninfected HT-1080 control cells revealing equivalent pharmacology between IncuCyte® Nuclight™ Green BacMam 3.0 labeled and non-transduced cells.

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<th>Promoter</th>
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<th>Amount</th>
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