IncuCyte™ NucLight™ BacMam 3.0 Frequently Asked Questions

Q: Will the NucLight™ BacMam 3.0 reagent cause toxicity in my cells?

A: The NucLight™ BacMam 3.0 reagent induces little to no cytotoxic effects in the range of cell types tested. For each cell type being tested, we recommend a transduction optimization experiment in order to evaluate expression level and toxicity.

Q: What cell types can be used with the NucLight™ BacMam 3.0 reagent?

A: The NucLight™ BacMam 3.0 has been shown to work with a broad range of cell types including; immortalized cell lines, primary cells such as neurons, and stem cell lines. The NucLight™ BacMam 3.0 reagent is not recommended for primary T cells.

Q: When should I use the NucLight™ BacMam 3.0 reagent instead of the NucLight™ Lentivirus reagent?

A: The NucLight™ BacMam 3.0 reagent offers an effective alternative for gene delivery into mammalian cells, when vector safety and genome capacity are of concern. This methodology enables delivery of a label in to cells just prior to assay initiation without the need to establish fluorescently stable cell line(s). Alternatively, the NucLight™ Lentivirus reagent can be used when the generation of a stable cell line is necessary.

Q: When will I start to see nuclear expression?

A: Expression can usually be seen 6 hours following infection, however peak expression is typically observed after around 20 hours. We recommend waiting at least 20 hours after infection before analyzing nuclear expression in the IncuCyte ZOOM® live-cell imaging system.

Q: How long does expression last?

A: The duration of nuclear fluorescent protein expression depends on a range of factors, including the amount of NucLight™ BacMam 3.0 reagent used, the doubling time of the cells, and the transduction efficiency in the cell type. In most dividing cell types, expression will last longer than 72 hours, but in non-dividing cell lines, such as neurons, expression can last for more than 2 weeks.

Q: What is the transduction efficiency?

A: We have found the transduction efficiency to be greater than 80% in all of the cell types we have tested. However, transduction efficiency depends on many factors, such as cell type, the amount of virus being used, and the cell density at the time of transduction.

Q: Does the NucLight™ BacMam 3.0 reagent need to be removed after infection?

A: No. The NucLight™ BacMam 3.0 reagent can be left in the media with cells throughout your experiment. We only recommend removing the virus when performing compound additions due to convenience.

Q: What concentration of the NucLight™ BacMam 3.0 reagent should I use in my assay?

A: Generally, a range of 0.5 to 2% v/v of the NucLight™ BacMam 3.0 reagent is recommended for seeding densities of 1,000 or 2,000 cells per well. We recommend optimization for each cell type being used, keeping in mind that transduction efficiency is effected by cell confluence.

Q: Can I create a stable cell line using the NucLight™ BacMam 3.0?

A: No. The NucLight™ BacMam 3.0 virus does not integrate into the mammalian genome, therefore protein expression is transient. Our NucLight™ Lentivirus reagents are recommended for generating stable cell lines.

Q: Is the NucLight™ BacMam 3.0 safe to use?

A: Yes. The NucLight™ BacMam 3.0 virus is an insect virus and is non-replicating in mammalian cells. It can be handled safely under BSL 1 conditions.

Q: Can I store the NucLight™ BacMam 3.0 reagents at -20°C?

A: No. Storing NucLight™ BacMam 3.0 reagents at temperatures below 4°C will greatly reduce your infectious titer, and therefore, transduction efficiency will decrease significantly.

Q: What happens if I leave the NucLight™ BacMam 3.0 at ambient temperature?

A: Performance of the NucLight™ BacMam 3.0 reagent should be unaffected even after a few days at ambient temperature however we recommend storage at 4°C to maintain stability.

Q: Do I need to incubate my cells at ambient temperature for 20 min following NucLight™ BacMam 3.0 infection?

A: Incubation at ambient temperature greatly enhances the NucLight™ BacMam 3.0 reagent expression and is an important step that should not be overlooked.

Q: Can I transduce cell that have already been plated using the NucLight™ BacMam 3.0 reagent?

A: Yes this is possible however if your cells that have already adhered to the plate surface we recommend performing a full media change when adding the NucLight™ BacMam 3.0 reagent to maximize protein expression.

Q: Can I multiplex the NucLight™ BacMam 3.0 reagent with other live-cell reagents?

A: Yes. Multiplexed measurements of cell health can be made using any unused imaging channel. The IncuCyte ZOOM® live-cell imaging instrument supports High Definition phase-contrast, green fluorescence and red fluorescence automated imaging modes.

Essen BioScience, 300 West Morgan Road., Ann Arbor, Michigan, USA 48108