molecular probes®
by life technologies™

Premo™ FUCCI Cell Cycle Sensor *BacMam 2.0*

Catalog nos. P36237, P36238

Table 1 Contents and storage

Material	Amount	Concentration	Storage*	Stability
Premo™ geminin-GFP (G2/M reagent) (Component A)	1 mL (P36237) or 200 µL (P36238)	~1 × 10^8 viral particles/mL	• 2–8°C • Desiccate • Protect from light • DO NOT FREEZE	When stored as directed, this kit is stable for at least 6 months.
Premo™ Cdt1-RFP (G1/S reagent) (Component B)	1 mL (P36237) or 200 µL (P36238)	~1 × 10^8 viral particles/mL		
*These storage conditions are appropriate when storing the entire kit upon receipt. After preparing stock solutions, optimal storage conditions may change. For storing prepared stock solutions, follow recommendations included in this product information sheet.				
Number of assays: Sufficient material is supplied for 50–100 coverslips, based on the protocol below.				
Approximate fluorescence excitation/emission maxima: Premo™ geminin-GFP: 485/520 nm; Premo™ Cdt1-RFP: 555/584 nm.				

Introduction

In 2008, Miyawaki and colleagues developed the Fluorescence Ubiquitination Cell Cycle Indicator (FUCCI), a fluorescent protein (FP)-based sensor that employs a red (RFP) and a green (GFP) fluorescent protein fused to different regulators of the cell cycle: Cdt1 and geminin.[1] These two constructs, Cdt1 and geminin, are ubiquitinated by specific ubiquitin E3 ligases targeting them to the proteasome for degradation. The temporal regulation of the activity of these E3 ligases results in the biphasic cycling of geminin and Cdt1 through the cell cycle. In the G1 phase of the cell cycle, geminin is broken down and only Cdt1 tagged with RFP may be visualized, thus identifying cells in the G1 phase with red fluorescent nuclei. In the S, G2, and M phases, however, Cdt1 is degraded and only geminin tagged with GFP remains, thus identifying cells in these phases with green fluorescent nuclei. During the G1/S transition, as Cdt1 levels decrease and geminin levels increase, both proteins are present in the cells, allowing GFP and RFP fluorescence to be observed—when green and red images are overlaid, the cells appear with yellow fluorescent nuclei. This dynamic color change from red-to-yellow-to-green represents the progression through cell cycle and division (Figures 1 and 2, next page).

The Premo™ FUCCI Cell Cycle Sensor combines the Cdt1 and geminin FP constructs with the powerful BacMam gene delivery system. The genetically encoded and pre-packaged reagents enable immediate usage and eliminate the need to purify plasmid or to use lipids, dye-loading chemicals, or other potentially harmful treatments to transduce cells. Additionally, BacMam technology permits defined optimization as expression levels can easily be titrated by adding more or less virus to cells in culture. Cellular transduction is efficient and reproducible in most cell types, including primary and stem cells, without apparent cytotoxic effects. BacMam 2.0 greatly expands the efficiency and utility of this

popular gene delivery platform.[2–4] Cell types previously not compatible with the technology (primary neurons), or cells that were poorly transduced with version 1.0 (some stem cells, CHO) can now be transduced quantitatively in a simple, one-step process. The improved performance is due to inclusion of elements that greatly enhance transduction efficiency and expression levels: a pseudotyped capsid protein for more efficient cell entry and genetic elements (enhanced CMV promoter and Woodchuck Post-Transcriptional Regulatory Element) that boost expression levels. To date, over 90 cell types have been shown to be effectively transduced using BacMam delivery technology. For the most up-to-date list of cells and transduction efficiencies, visit www. lifetechnologies.com/bacmamcompatible.

Premo™ FUCCI Cell Cycle Sensor is designed for live-cell imaging of cell cycle progression and can be used to assess the effect of drugs, siRNA, or other factors on the transition of cells through the cell cycle. The fluorescence from geminin-GFP and Cdt1-RFP have been demonstrated to be resistant to fixation with 4% formaldehyde and permeabilization with 0.1% Triton® X-100, thereby enabling processing of labeled cells with antibodies to other cellular targets. Each kit contains all of the components needed to label cells with the Premo™ FUCCI Cell Cycle Sensor using a transduction volume of 1 mL; however, the protocol can easily be adjusted for larger or smaller volumes. The workflow is straightforward: just add the reagent directly to your cells and incubate overnight to allow for the expression of fluorescent proteins. The next day, visualize cell cycle progression in populations of cells using traditional fluorescence microscopy (Figure 3, next page).

Figure 1 Dynamic color change of the Premo™ FUCCI Cell Cycle Sensor

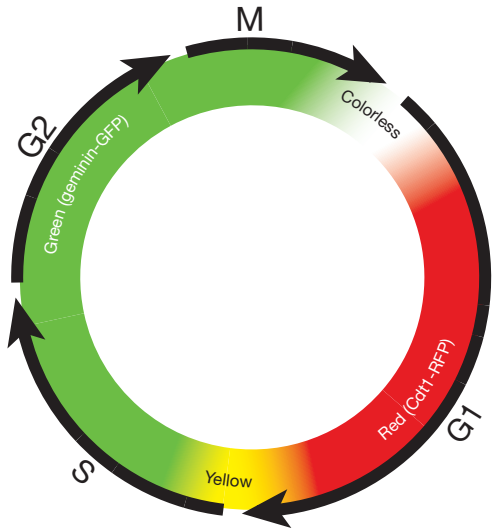


Figure 2 Fluorescence excitation and emission spectra for Premo™ geminin-GFP (panel A) and Premo™ Cdt1-RFP (panel B)

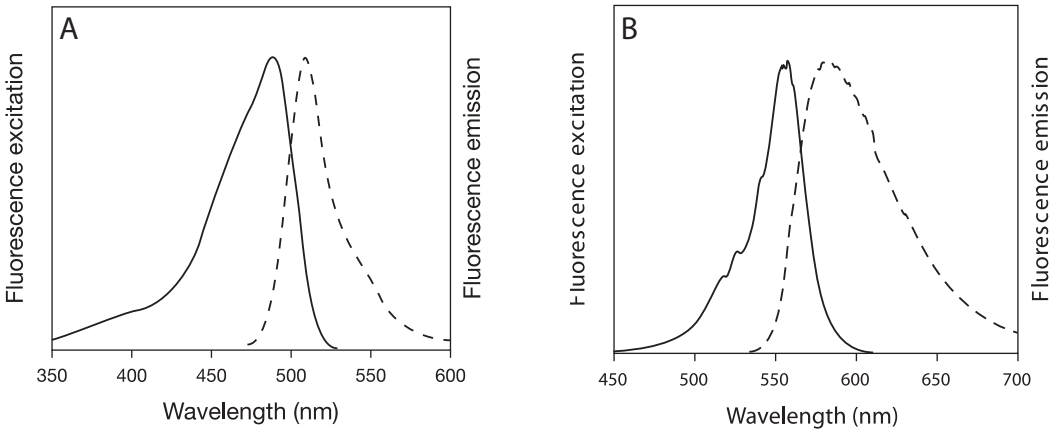
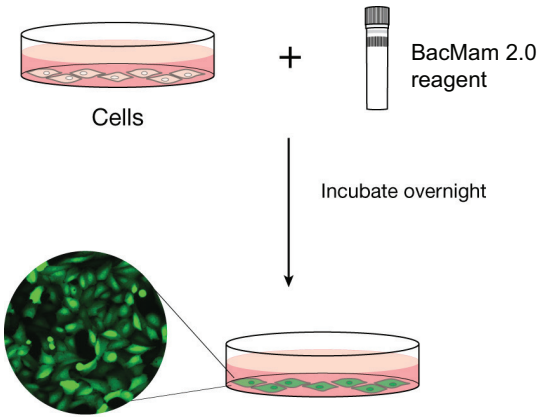


Figure 3 Workflow for Premo™ FUCCI Cell Cycle Sensor *BacMam 2.0*



Before Starting

Working with Premo™ FUCCI Cell Cycle Sensor Reagents

- Premo™ FUCCI Cell Cycle Sensor reagents work with most cell types between 20 and 100 particles per cell (PPC).
- For best results, transduce cells at a confluence of no more than 70%.
- The BacMam Enhancer (Cat. no. B10107) is generally not required for BacMam 2.0 reagents. However, its use has been shown to boost expression in some challenging cell types such as Jurkat.
- For optimal results you may need to alter the PPC, volume, cell density, temperature, or incubation time. Following the PPC, adjusting the volume is the next best parameter to change to optimize protein expression.

Experimental Protocols

Labeling Cells with Premo™ FUCCI Cell Cycle Sensor

The following protocol is based on a 1 mL labeling volume and ~50,000 cells plated in a 35-mm dish or for 1 well of a 6-well culture plate and a PPC (particles per cell) of 40. For applications that require a larger number of cells such as flow cytometry and high-content screening (HCS), we recommend plating cells in a 10-cm dish or T-75 flask and increasing the labeling volume to 5 mL with a proportionate increase in the volume of the virus.

1.1 Plate cells at a desired density and allow sufficient time for cells to adhere. BacMam reagents work best when used on cells of a low passage number plated at a low density. Plating density should take into account the desired confluence at the time of cell labeling and accommodate additional growth during the overnight incubation required for expression of geminin-GFP and Cdt1-RFP.

1.2 Calculate the volume of Premo™ geminin-GFP (Component A) and Premo™ Cdt1-RFP (Component B) using the equation below.

$$\text{Volume of Premo™ geminin-GFP or Premo™ Cdt1-RFP reagent (mL)} = \frac{(\text{number of cells})(\text{PPC})}{(1 \times 10^8)}$$

where the number of cells is the estimated total number of cells at the time of cell labeling, PPC (particles per cell) is the number of viral particles per cell, and 1×10^8 is the number of viral particles per mL of the reagent.

For example, to label 50,000 cells with a PPC of 40:

$$\text{mL of Premo™ geminin-GFP or Premo™ Cdt1-RFP reagent} = \frac{(50{,}000)(40)}{(1 \times 10^8)} = 0.02 \text{ mL } (20 \text{ µL})$$

Note: Sakaue-Sawano, *et. al.* report no deleterious effects of these modified proteins on the cell cycle;[1] however, we encourage optimizing the experimental conditions to avoid overexpressing geminin-GFP and Cdt1-RFP. Factors that affect labeling efficiency include PPC, labeling volume, and the incubation time. We have found an PPC of 40 to be a good starting point for expression studies. Table 2 provides initial guidelines for these factors; however, we recommend that you optimize for your specific cell type and application.

Table 2 Initial guidelines and factors for optimization of Premo™ FUCCI Cell Cycle Sensor labeling efficiency

Cell type	PPC	Labeling volume
Standard cell lines (HeLa, A549, CHO)	40	1 mL
Cell lines that are difficult to transfect	80	0.5 mL
	40	0.5 mL

1.3 Mix each Premo™ reagent (Components A and B) by inversion to ensure a homogenous solution.

1.4 Add the volume of Premo™ reagent calculated in step 1.2 directly to the cells in complete cell medium and mix gently.

1.5 Return the cells to the culture incubator overnight (≥16 hours).

Imaging and Analysis

Note: At this stage, you may trypsinize the cells and store frozen at –80°C or in liquid nitrogen for future use.

2.1 Image and analyze using appropriate instrument filter sets. Refer to Figure 2 (page 2) for spectral characteristics of geminin-GFP and Cdt1-RFP.

Premo™ FUCCI Cell Cycle Sensor is primarily designed for live-cell imaging of cell cycle progression, but the fluorescence from geminin-GFP and Cdt1-RFP have been demonstrated to be resistant to fixation with 4% formaldehyde and permeabilization with 0.1% Triton® X-100, enabling processing of labeled cells with antibodies to other cellular targets. You can accurately correlate Premo™ FUCCI Cell Cycle Sensor expression with cell cycle phase by measuring DNA content. For live cell imaging, the cell-permeant nucleic acid stains Hoechst 33342 and HCS NuclearMask™ Blue stains are spectrally compatible with the Premo™ FUCCI Cell Cycle Sensor fluorescence.

Frequently Asked Questions

Q: Will BacMam 2.0 transduce my cells?

A: The first generation BacMam reagents were shown to efficiently transduce over 90 cell types, including stable cell lines and primary cells. For the most up-to-date list of cells and transduction efficiencies, refer to www.lifetechnologies.com/bacmamcompatible. With BacMam 2.0, it is now possible to efficiently transduce primary neurons and stem cells.

Q: How long does expression last?

A: The duration of transgene expression depends on many factors, including transduction levels, cell division rates, mRNA, and protein stability. In most transformed cell lines, such as HeLa and CHO, expression lasts about 5 days. In cells that divide more slowly or show contact inhibition, such as some stem cells, primary cells, and neurons, we have observed bright staining and transgene expression for more than 2 weeks. For non-dividing, terminally differentiated cells we have observed expression for 2 to 4 weeks.

Q: Can I transduce with more than one BacMam reagent at a time?

A: Yes, this is one of the advantages of the system. For instance the Premo™ FUCCI Cell Cycle Sensor and the BacMam Kv7.2/7.3 Potassium Ion Channel reagent are based on optimized ratios of 2 BacMam constructs that give rise to a two-color cell cycle sensor and a functional heterotetrameric K channel, respectively.

Q: Will BacMam transduction hurt my cells?

A: BacMam transduction is well-tolerated, even at very high number of viral particles to cell ratios (>1,000). However, we have occasionally observed apparent cytotoxic effects by some BacMam reagents at very high virus levels; this may be due to the nature of the transgene. For this reason, we recommend using no more virus than is needed.

Q: If I freeze my cells after transduction, how long can I store them without reducing expression levels?

A: Our data show that transduced cells can be stored in liquid nitrogen for several months without reducing the level of transgene expression.

Q: Can transduction be optimized if my cells are difficult to transduce?

A: Yes. Try varying particle-to-cell ratio (PPC), incubation volume, temperature or duration, and cell density (if adherent cells are transduced). For adherent cells, we recommend a confluence of about 70%. Following the PPC, adjusting the volume is the next best parameter to change to optimize protein expression.

Q: Can a cell be transduced more than once?

A: Yes. Because transduction is well-tolerated, you can add more BacMam reagent after a few days if expression levels need to be boosted or if a different BacMam-based assay is needed.

Q: It's a virus—is it safe to use?

A: Yes. Baculoviruses are insect viruses that do not replicate in mammalian cells and are generally used under the safety precautions common for standard cell-based reagents.

References

1. Cell 132, 487 (2008); **2.** Biochem Biophys Res Comm 349, 1220 (2006); **3.** J Biotechnol 131, 1 (2007); **4.** Mol Ther 17, 1585 (2009).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
P36237	Premo™ FUCCI Cell Cycle Sensor *BacMam 2.0* *set of 2 vials*	1 mL set
P36238	Premo™ FUCCI Cell Cycle Sensor *BacMam 2.0* *set of 2 vials*	200 µL set
Related Products		
B10107	BacMam Enhancer Kit	1 kit
H3570	Hoechst 33342, trihydrochloride, trihydrate *10 mg/mL solution in water*	10 mL
H10325	HCS NuclearMask™ Blue stain *for 10 × 96-well plates* *2000X concentrate*	65 µL

Purchaser Notification

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