

Premo™ FUCCI Cell Cycle Sensor

Catalog no. P36232

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage*	Stability
Premo™ geminin-GFP (G2/M reagent) (Component A)	0.5 mL	$\sim 1 \times 10^8$ viral particles/mL	<ul style="list-style-type: none"> • 2–6°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)	0.5 mL	$\sim 1 \times 10^8$ viral particles/mL		
BacMam enhancer (Component C)	1 vial	NA		
Dimethylsulfoxide (DMSO) (Component D)	100 µL	NA	<ul style="list-style-type: none"> • $\leq 25^\circ\text{C}$ • Desiccate 	

*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. NA = Not applicable.

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).

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. To date, over 90 cell types have been shown to be effectively transduced using BacMam delivery technology, including stable cell lines and primary cells. Currently BacMam delivery does not work well for hematopoietic or macrophage cells. For the most up to date list of cells and transduction efficiencies, refer to www.invitrogen.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 2 mL; however, the protocol can easily be adjusted for larger or smaller volumes. The workflow is straightforward: just add the reagents to your cells for 1–2 hours and then treat with the BacMam enhancer (included in the kit) for 1–2 hours. After a wash and overnight incubation to allow for the expression of fluorescent proteins, cell cycle progression in populations of cells can be visualized using traditional fluorescence microscopy (Figure 3).

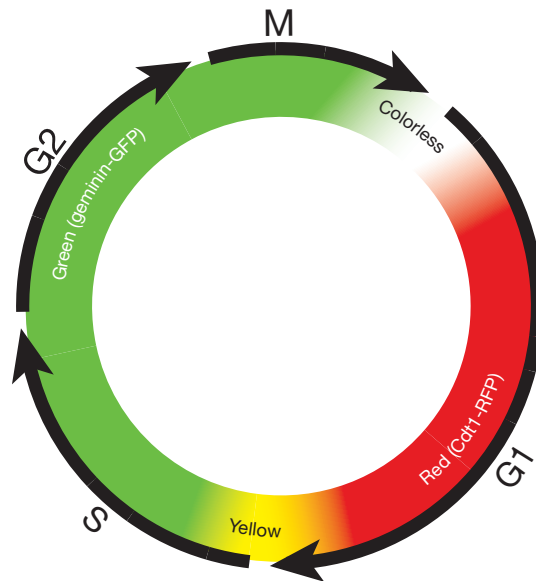


Figure 1. Premo™ FUCCI Cell Cycle Sensor schematic.

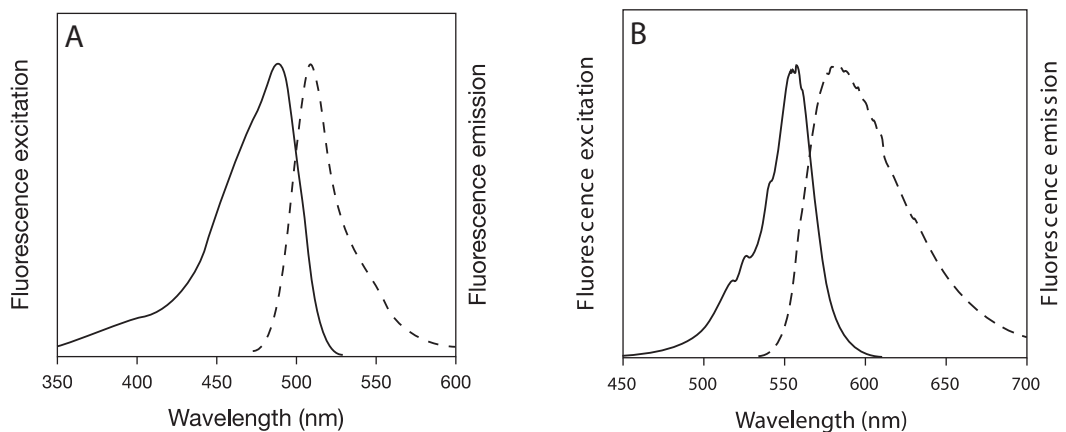


Figure 2. Fluorescence excitation and emission spectra for Premo™ geminin-GFP (panel A) and Premo™ Cdt1-RFP (panel B).

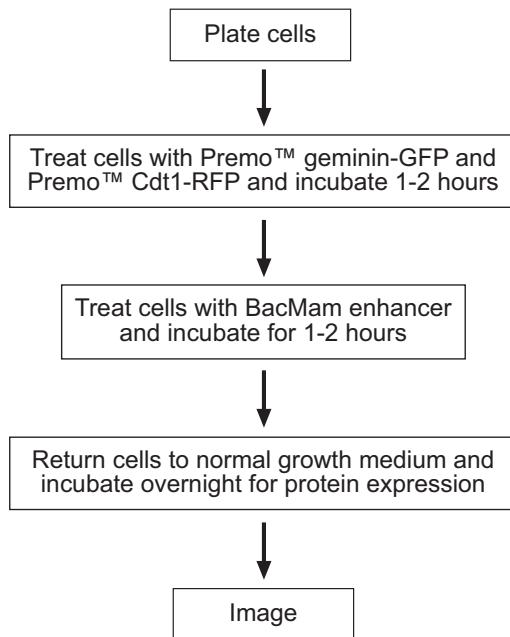


Figure 3. Workflow diagram for Premo™ FUCCI Cell Cycle Sensor.

Before Starting

Materials Required but Not Provided

Dulbecco's buffered saline (D-PBS) without calcium or magnesium.

Caution

BacMam enhancer (Component C) may cause sensitization by skin contact, and is harmful by inhalation and if swallowed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable laboratory protective clothing and gloves while handling this reagent.

DMSO (Component D) is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of the reagents in compliance with all pertaining local regulations.

Preparing the BacMam Enhancer Stock Solution

- 1.1 Prepare a 1,000X BacMam enhancer solution by dissolving the entire contents of the BacMam enhancer (Component C) in 100 μ L of DMSO (Component D). Aliquot and store any remaining stock solution at 2–6°C, **protected from light**. When stored as directed, this stock solution is stable for up to 6 months.

Experimental Protocols

Labeling Cells with Premo™ FUCCI Cell Cycle Sensor

The following protocol is based on a 2 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 MOI (multiplicity of infection) of 20. 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 10 mL with a proportionate increase in the volume of the virus.

- 2.1 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.
- 2.2 Calculate the volume of Premo™ geminin-GFP (Component A) and Premo™ Cdt1-RFP (Component B) using the equation below.

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

where the number of cells is the estimated total number of cells at the time of cell labeling, MOI (multiplicity of infection) 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 MOI of 20:

$$\text{mL of Premo™ geminin-GFP or Premo™ Cdt1-RFP reagent} = \frac{(50,000)(20)}{(1 \times 10^8)} = 0.01 \text{ mL (10 } \mu\text{L)}$$

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 MOI, labeling volume, and the incubation time. We have found an MOI of 20 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.

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

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

Cell type	MOI	Labeling volume	Incubation time
Standard cell lines (HeLa, A549, CHO)	20	2 mL	120 minutes
Primary cells and cells sensitive to lack of divalent ions	40	1 mL	30–60 minutes
	20	1 mL	

- 2.4 Prepare the Premo™ FUCCI transduction solution by adding 10 μL each of the Premo™ geminin-GFP and Premo™ Cdt1-RFP reagents to 2 mL of D-PBS without calcium or magnesium in a sterile tube.
- 2.5 Remove cell culture media from cells and add the Premo™ FUCCI transduction solution (prepared in step 2.4) to each dish or well.

- 2.6 Incubate at room temperature for 2 hours with gentle rocking. You may incubate cells that detach due to a lack of calcium and magnesium for a shorter period; however, an increased MOI or a decreased labeling volume may be necessary to compensate for the decreased incubation time (see Table 2 for guidelines).
- 2.7 While cells are incubating with the Premo™ FUCCI transduction solution, prepare the 1X BacMam enhancer working solution by diluting the 1,000X BacMam enhancer stock solution (prepared in step 1.1) 1:1,000 in complete media (*i.e.*, add 2 µL of 1,000X BacMam enhancer stock solution to 2 mL of complete media).
- 2.8 Remove Premo™ FUCCI transduction solution and add the 1X BacMam enhancer working solution (prepared in step 2.7) to each dish or well. Incubate for 60–90 minutes under normal growth conditions.
- 2.9 Remove 1X BacMam enhancer working solution and replace with normal growth medium. Return cells to normal growth conditions for ≥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.

- 3.1 Image and analyze using appropriate instrument filter sets. Refer to Figure 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.

Reference

1. Cell 132, 487 (2008).

Product List

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

Cat. no.	Product Name	Unit Size
P36232	Premo™ FUCCI Cell Cycle Sensor	1 kit
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
14190-136	Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X) liquid (contains no calcium or magnesium)	1,000 mL
14190-250	Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X) liquid (contains no calcium or magnesium)	10 × 500 mL
14190-359	Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X) liquid, Universal Bag (contains no calcium or magnesium).....	10 L

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