molecular probes® by life technologies™

CellLight® Reagents *BacMam 2.0*

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Table 1 Contents and storage

Material	Amount*	Storage	Stability
CellLight® Reagent *BacMam 2.0*	1 mL or 100 µL	• 2˚C to 8˚C • Protect from light • DO NOT FREEZE	When stored as directed, the product is stable for 3 months.
Approximate fluorescence excitation/emission maxima: CFP: 435/485 in nm; GFP: 485/520 in nm; RFP: 555/584 in nm.			
* Some of the CellLight® Reagents are available in 100 µL trial sizes. For more information, refer to the Product List on page 7.			

Introduction

CellLight® reagents are fluorescent protein-signal peptide fusions that provide accurate and specific targeting to cellular structures for live-cell imaging applications; they can also be used in fixed cell analyses following formaldehyde-based fixation. Cellular labeling utilizes BacMam technology, which uses an insect cell virus (baculovirus) coupled with a mammalian promoter.[1,2] Transgenes under the mammalian promoter are expressed, while baculoviral genes and their promoters are not recognized. The inability of baculoviruses to replicate in mammalian cells renders them safe as research reagents and provides a transient footprint-free method to label cells.

The CellLight® reagents are ready for immediate use – there is no need to purify plasmid or be concerned about vector integrity and quality. No lipids, dye-loading chemicals, or potentially harmful treatments are required. Unlike conventional stains, CellLight® reagents stain independently of function (i.e., membrane potential). To use, simply add the reagent to your cells, incubate overnight for protein expression, then visualize (see Figure 1). Unlike expression vectors, BacMam reagents enable titratable and reproducible expression, and offer high co-transduction efficiency; therefore, multiple BacMam reagents can readily be used in the same cell.

BacMam 2.0 greatly expands the efficiency and utility of this popular gene delivery platform.[3–5] 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**.

CellLight® reagents contain a signal peptide or protein fused to CFP, emerald GFP (emGFP), or TagRFP (see Table 2, Figure 3). The CellLight® Null (control) reagent lacks any mammalian genetic elements and can be used to help determine potential baculovirus-mediated effects and background fluorescence. BacMam 2.0 can also be evaluated with the BacMam 2.0 GFP transduction control (Cat. no. B10383), a non-targeted form that will light up the entire cell.

Figure 1 BacMam 2.0 workflow

Using the BacMam 2.0 to express genes is simple and efficient:
1. Add the BacMam reagent directly to the cells.
2. Analyze the transduction efficiency the next day or freeze the cells for future use.

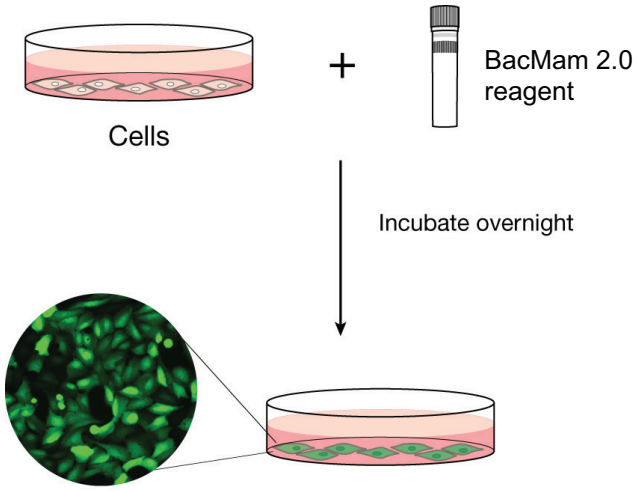


Figure 2 Comparison of the transduction efficiency of BacMam 1.0 and BacMam 2.0 using the BacMam GFP transduction control (Cat. no. B10383)

Panels A1 and B1 show BacMam 1.0 in T84 adenocarcinoma and adipocyte-derived stem cells (ADSC), respectively, while panels A2 and B2 show BacMam 2.0 in T84 adenocarcinoma and adipocyte-derived stem cells (ADSC), respectively. Transduction conditions including virus titer, particles per cell, treatment time, and cell density were identical.

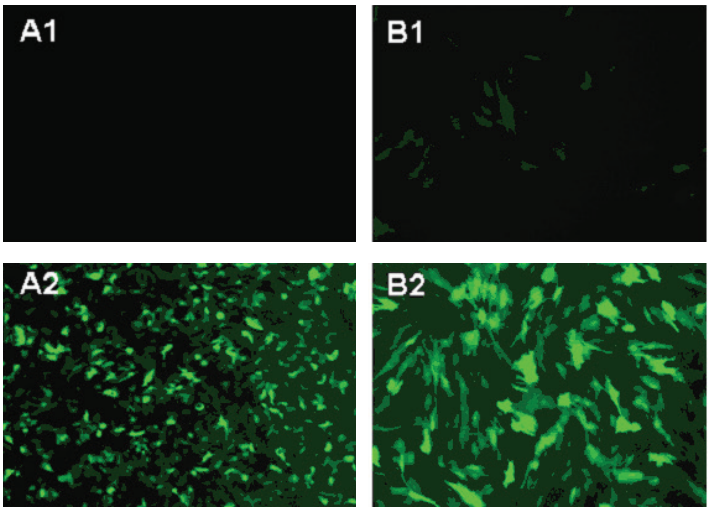
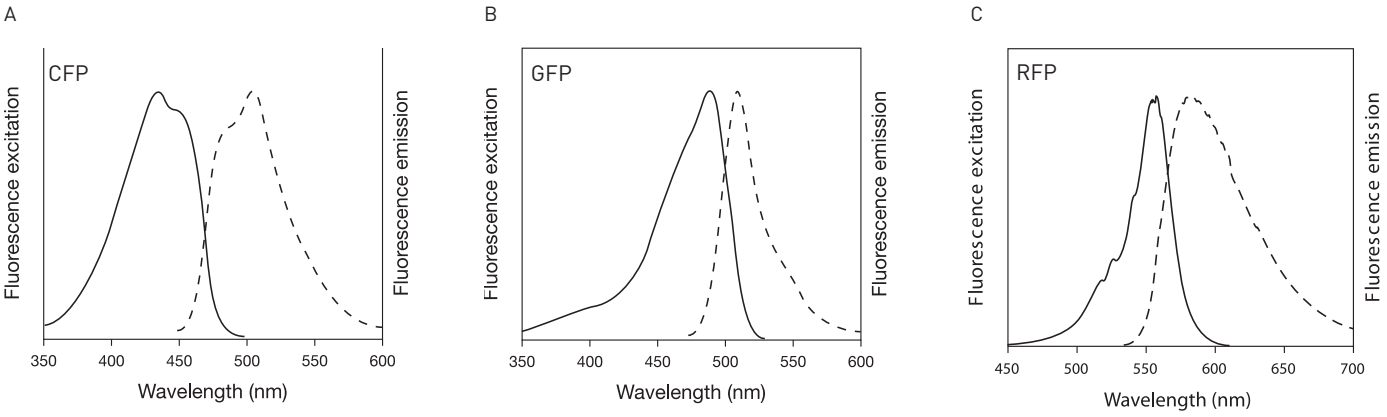


Table 2 CellLight® targeting information and available colors

Structure	Targeting sequence	Reference	CFP*	GFP*	RFP*
Actin	Human actin	6, 7		X	
Early endosomes	Rab5a	8		X	X
Late endosomes	Rab7a	21			X
Endoplasmic reticulum (ER)	ER signal sequence of calreticulin and KDEL (ER retention signal)	9		X	X
Golgi	Human Golgi-resident enzyme N-acetylgalactosaminyltransferase 2	10		X	X
Histones	Histone 2B	11		X	X
Lysosomes	Lamp1 (lysosomal associated membrane protein 1)	12		X	X
MAP4	MAP4	13		X	X
Mitochondria	Leader sequence of E1 alpha pyruvate dehydrogenase	14		X	X
Nucleus	SV40 nuclear localization sequence	15	X	X	X
Peroxisomes	Peroxisomal C-terminal targeting sequence	16		X	X
Plasma membrane	Myristolyation/palmitoylation sequence from Lck tyrosine kinase	17	X	X	X
Synaptic vesicles	Synaptophysin	18			X
Talin	Human c-terminus of talin	19		X	X
Tubulin	Human tubulin	20		X	X

*CFP: Cyan Fluorescent Protein; GFP: Green Fluorescent Protein; RFP: Red Fluorescent Protein.

Figure 3 Fluorescence excitation and emission spectra for CellLight® Cyan Fluorescent Protein (CFP, panel A), CellLight® Green Fluorescent Protein (emerald GFP, panel B), and CellLight® Red Fluorescent Protein (TagRFP, panel C), obtained in PBS, pH 7.4 (for GFP and CFP). The RFP spectra was determined in 100 mM NaCl, 20 mM Tris-HCl, pH 7.5.



Before starting

CellLight® reagents

- CellLight® reagents work with most cell types between 10 and 50 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

CellLight® reagents are provided as 1×10^8 particles/mL solution. The following protocol was optimized using adherent cells. Cells can also be labeled in suspension prior to plating.

Day 1: Labeling

1.1 Plate the cells at the desired density and allow them sufficient time to adhere. BacMam reagents work best when used on low-passage-number cells.

1.2 Calculate the appropriate volume of CellLight® reagent for the number of cells.

$$\text{Volume of CellLight® Reagent (mL)} = \frac{\text{number of cells} \times \text{desired PPC}}{1 \times 10^8 \text{ CellLight® particles/mL}}$$

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

For example, to label 40,000 cells with a PPC of 30:

$$\text{Volume of CellLight® Reagent (mL)} = \frac{40{,}000 \times 30}{1 \times 10^8} = 0.012 \text{ mL (12 µL)}$$

1.3 Mix the CellLight® reagent several times by inversion to ensure a homogenous solution. Do not vortex.

1.4 Add the volume of CellLight® 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).

Day 2: Imaging

2.1 Image the cells using the appropriate instrument filter sets. Refer to Figure 3 for spectral characteristics of CFP, GFP, and RFP.

Optional: You may fix your cells with formaldehyde. To fix cells, treat with 4% formaldehyde solution in PBS for 10–30 minutes at room temperature. You can permeabilize the cells following fixation with 0.2% Triton® X-100 solution in PBS for 5 minutes at room temperature.

Note: Fluorescence may be lost with methanol fixation. If methanol fixation is necessary for downstream analyses, you may detect CFP and GFP proteins using an anti-GFP antibody; RFP can be detected with an anti-RFP antibody. These antibodies are available separately. Go to **www.lifetechnologies.com**.

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. Nature Biotechnol 23, 567 (2005); **2.** Expert Opin Drug Discov 2, 1669 (2007); **3.** Biochem Biophys Res Comm 349, 1220 (2006); **4.** J Biotechnol 131, 1 (2007); **5.** Mol Ther 17, 1585 (2009); **6.** Nature Methods 4, 555 (2007); **7.** Curr Biol 7, 176 (1997); **8.** J Biol Chem 284, 29218 (2009); **9.** FEBS Letters 405, 18 (1997); **10.** J Cell Biol 143, 1505 (1998); **11.** Curr Biol 8, 377 (1998); **12.** J Cell Sci 118, 5243 (2005); **13.** J Cell Biol 130, 639 (1995); **14.** J Biol Chem 279, 13044 (2004); **15.** TiBS 16, 478 (1991); **16.** J Cell Biol 108, 1657 (1989); **17.** EMBO 16, 4983 (1997); **18.** J Neuroscience 26, 3604 (2006); **19.** Plant J 33, 775 (2003); **20.** PLos ONE 4, e8171 (2009); **21.** J Biol Chem 284, 29218 (2009).

Product list Current prices may be obtained at **www.lifetechnologies.com.**

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	Cat. no.	Product name	Unit size
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Buy Now	C10506	CellLight® Actin-GFP *BacMam 2.0*	100 µL
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Buy Now	C10596	CellLight® Lysosomes-GFP *BacMam 2.0*	1 mL
Buy Now	C10507	CellLight® Lysosomes-GFP *BacMam 2.0*	100 µL
Buy Now	C10597	CellLight® Lysosomes-RFP *BacMam 2.0*	1 mL
Buy Now	C10504	CellLight® Lysosomes-RFP *BacMam 2.0*	100 µL
Buy Now	C10598	CellLight® MAP4-GFP *BacMam 2.0*	1 mL
Buy Now	C10599	CellLight® MAP4-RFP *BacMam 2.0*	1 mL
Buy Now	C10600	CellLight® Mitochondria-GFP *BacMam 2.0*	1 mL
Buy Now	C10508	CellLight® Mitochondria-GFP *BacMam 2.0*	100 µL
Buy Now	C10601	CellLight® Mitochondria-RFP *BacMam 2.0*	1 mL
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Buy Now	C10614	CellLight® Tubulin-RFP *BacMam 2.0*	1 mL
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Buy Now	G10362	GFP, ABfinity™ recombinant rabbit monoclonal antibody - unconj (anti-GFP, rabbit mAb)	100 µg
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Buy Now	R10367	RFP, rabbit polyclonal antibody - unconjugated	100 µg

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