BacMam GFP Transduction Control *BacMam 2.0*

Catalog no. B10383

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
BacMam GFP Transduction Control *BacMam 2.0*	1 mL	~1 × 10^8 particles/mL	• 2–6°C • Protect from light • DO NOT FREEZE	When stored as directed, this kit is stable for 6 months.
Approximate fluorescence excitation/emission maxima: GFP**:** 488/512 in nm.				
Number of assays: Sufficient material is supplied to transduce approximately 5 × 10^6 cells based on the protocol below.				

Introduction

The BacMam GFP Transduction Control lets you experience the power and convenience of the enhanced BacMam 2.0 technology, with a convenient readout of emerald GFP (emGFP). The extremely bright and photostable *Aequorea victoria* emGFP lights up the entire cell in this non-targeted form. Evaluate BacMam 2.0 in your cell model before advancing to the more specialized BacMam reagents.

BacMam technology is based on the use of an insect cell virus (baculovirus) to efficiently deliver and express genes in mammalian cells.[1,2] Transgenes under mammalian promoter elements are expressed, while baculoviral genes and their promoters are not recognized. As mammalian cells do not support replication of baculoviruses, transduction is extremely well tolerated and generally lacking in cytopathic effects, even at high virus levels. The inability of baculoviruses to replicate renders them safe as research reagents.

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.

For more information on a range of BacMam-based reagents, including CellLight™ cell labeling reagents, Premo™ Biosensors, ion channel drug targets, and pathway analysis kits that facilitate the study of live cells, visit www.invitrogen.com/bacmam.

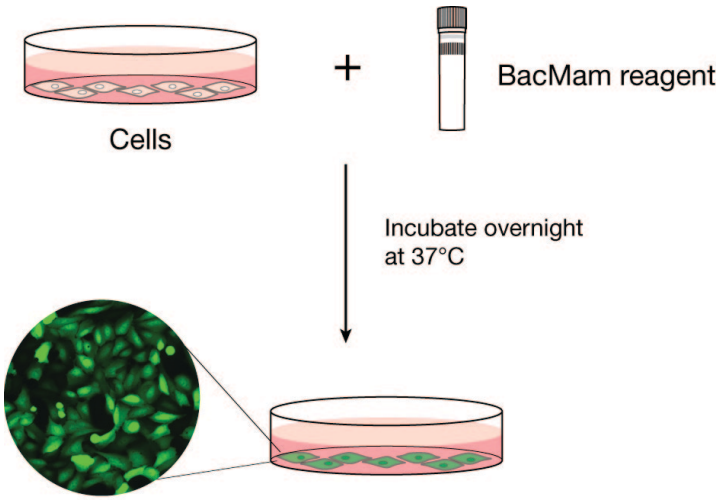

Figure 1. BacMam 2.0 workflow. Using the BacMam 2.0 to express genes is as simple as it is efficient:
1. Add the BacMam reagent directly to the cells.
2. Analyze the transduction efficiency the next day or freeze the cells for future use.

Table 2. Generalized comparison of the transduction efficiencies of BacMam 1.0 and BacMam 2.0 across different cell types. Gene expression can be increased by using the BacMam enhancer (Cat. no. B10107).

	BacMam 1.0 No enhancer	BacMam 1.0 With enhancer	BacMam 2.0 No enhancer	BacMam 2.0 With enhancer
Cell lines	+/–	++	++	++
Primary cells	+	++	++	++
Stem cells	–	++	++	++
Neurons	–	–	++	++
Immortalized T-cells	–	–	+/–	++
Primary T-cells and B-cells	–	–	–	–

– = not transduced
+/– = transduction efficiency ~10%
+ = transduction efficiency <50%
++ = transduction efficiency >50%

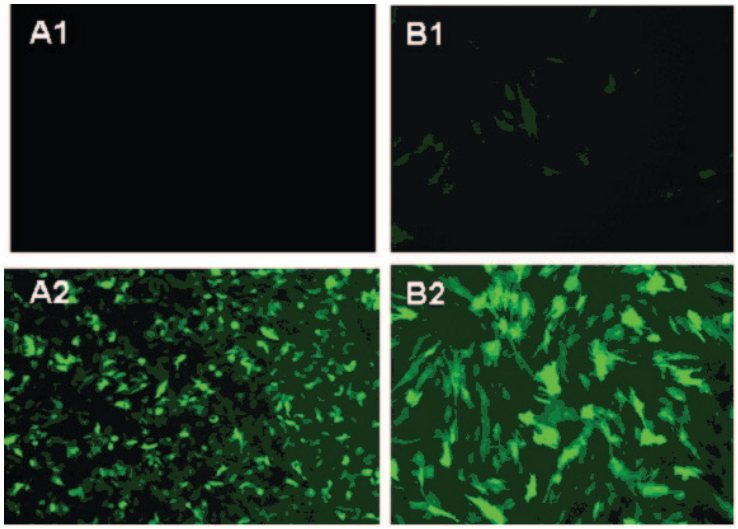

Figure 2. Comparison of the transduction efficiency of (1) BacMam 1.0 and (2) BacMam 2.0 GFP in T84 adenocarcinoma (A) and adipocytes-derived stem cells (ADSC) (B), respectively. Transduction conditions including virus titer, particles per cell, treatment time, and cell density were identical.

Before Starting

Materials Required but Not Provided

- Phosphate-buffered saline (PBS) without Ca^{2+}/Mg^{2+} (Cat. no. 10010)
- Hemacytometer and Trypan blue, or the Countess® Automated Cell Counter
- *Optional*: TrypLE™ Express dissociation enzyme (Cat. no. 12604-013)
- *Optional:* Freezing medium such as Recovery™ Cell Culture Freezing Medium (Cat. no. 12648-010)

Guidelines for Working with BacMam Reagents

- The standard protocol is based on a 1 mL labeling volume for a 35-mm dish or 1 well of a 6-well culture plate, with cells about 70% confluent at time of transduction, and 30 BacMam particles per cell.
- For applications that require a larger number of cells, such as high-content screening (HCS), we recommend transducing the cells in a 10-cm dish or a T-75 flask and increasing the labeling volume to 10 mL with a proportionate increase in the volume of the virus.
- Following an overnight incubation under normal growth conditions, trypsinize and count the cells for distribution to appropriate plates at the desired cell number.
- If the transduction efficiency needs to be optimized, we suggest adjusting the following variables: MOI (from 10 to 100), cell density (80,000 to 200,000 cells/mL), temperature (room temperature for 1 or 2 hours before moving to the incubator), transduction volume, and incubation time. For some very sensitive cell types a relatively high MOI for one or two hours followed by medium removal has also been reported to be effective.
- 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.
- We recommend transducing the cells at a confluence of about 70% for best results.
- For first time users of BacMam reagents, we recommend exceptionally well-transduced cells like U-2 OS (ATCC® Number: HTB-96™).

The BacMam GFP Transduction Control is provided as a 1×10^8 particles/mL solution. BacMam 2.0 reagents work best at a multiplicity of infection (MOI) between 10 and 50 in most cell types.

At a concentration of 1×10^8 particles/mL, for every 10,000 cells:

- 1 µL BacMam GFP Transduction Control is required for an MOI of 10
- 2 µL BacMam GFP Transduction Control is required for an MOI of 20
- 5 µL BacMam GFP Transduction Control is required for an MOI of 50

Note: Some cell types, such as neurons, may need a higher MOI.

Protocol for Adherent Cells

Day 1

1.0 Harvest cells for subculture in complete medium at the desired density, e.g. 200,000 cells/mL

1.1 Calculate the appropriate volume of BacMam GFP Transduction Control for the number of cells to be plated.

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

1.2 Mix the BacMam GFP Transduction Control reagent several times by inversion to ensure a homogenous solution. When using more than one BacMam reagent, pre-mix the reagents ahead of addition to the cells.

1.3 Add the appropriate volume of BacMam GFP Transduction Control reagent directly to the cells in complete cell medium and mix gently.

1.4 Incubate the cells at room temperature for 10 minutes.

1.5 Return the cells to the culture incubator and incubate overnight (≥16 hours) for GFP expression.

Note: Alternatively, add the BacMam GFP Transduction Control reagent to established cultures in complete medium at 1% to 10% (vol/vol), mix gently, and return to the incubator.

Day 2

1.6 Image the cells using the appropriate filters for GFP/FITC.

1.7 Replace medium if the cells are to be maintained in culture.

Notes:

- The BacMam GFP Transduction Control reagent was developed for use in live-cell studies. Should you prefer fixed cell analysis, the fluorescence from GFP has been shown to be compatible with fixation with 4% formaldehyde and permeabilization with 0.1% Triton® X-100.
- Increased transduction efficiencies are observed if the virus is added directly after plating, or while the cells are still in suspension.
- In most cases, the BacMam particles do not need to be removed, although a medium exchange the day following transduction may be desirable.
- Avoid exposing the BacMam reagent to light for more than 10 minutes.

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.invitrogen.com/BacMamCompatible. BacMam 2.0 GFP is based on a modified and enhanced BacMam vector with superior transduction properties. For instance, 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 two 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 two 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 generally exceptionally 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 at –80°C 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 virus-to-cell ratio (MOI), incubation temperature and duration, and cell density (if adherent cells are transduced). For adherent cells, we recommend a confluence of about 70%. Media have also been shown to affect transduction efficiency; if your cells do not tolerate the recommended PBS without Mg^{2+} and Ca^{2+}, we suggest using RPMI1640.

Q: Can a cell be transduced more than once?

A: Yes. Because transduction is so well tolerated, you can readily 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).

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

Cat. no.	Product Name	Unit Size
B10383	BacMam GFP Transduction Control *BacMam 2.0*	1 mL
Related Products		
A6455	anti-green fluorescent protein, rabbit serum (anti-GFP, serum)	100 µL
A11122	anti-green fluorescent protein, rabbit IgG fraction (anti-GFP, IgG) *2 mg/mL*	100 µL
B10019	BacMam-hERG *for 10 microplates* *BacMam 1.0*	1 kit
B10107	BacMam Enhancer Kit	1 kit
B10146	BacMam Kir2.1 *for 10 microplates* *BacMam 1.0*	1 kit
B10147	BacMam Kv7.2 and Kv7.3 *for 10 microplates* *BacMam 1.0*	1 kit
C10106	Cellular Lights™ Tubulin-GFP *BacMam 1.0*	1 kit
C10112	Cellular Lights™ Tubulin-RFP *BacMam 1.0*	1 kit
C10126	Cellular Lights™ Actin-GFP *BacMam 1.0*	1 kit
C10127	Cellular Lights™ Actin-RFP *BacMam 1.0*	1 kit
O10100	Organelle Lights™ Lysosomes-RFP *BacMam 1.0*	1 kit
O10104	Organelle Lights™ Endosomes-GFP *BacMam 1.0*	1 kit
O36210	Organelle Lights™ Mito-GFP *BacMam 1.0*	1 kit
O36231	Organelle Lights™ Endosomes-RFP *BacMam 1.0*	1 kit
P36232	Premo™ FUCCI Cell Cycle Sensor *BacMam 1.0*	1 kit
P36235	Premo™ Autophagy Sensor LC3B-GFP *BacMam 2.0*	1 kit
P36236	Premo™ Autophagy Sensor LC3B-RFP *BacMam 2.0*	1 kit

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