General Transduction Protocol

July 9, 2024

Product Info & Protocol

Introduction

The following protocol is a generalized BacMam transduction protocol. It is relevant for all Montana Molecular products that do not contain a fluorescent protein marker. As it covers a wide range of products, the suggested titration step on page 4 is important to determine optimal conditions for your experimental needs.

This protocol is optimized in rapidly dividing, immortalized cell lines on a 96-well plate, and has been validated in live HEK 293T cells [1]. For use in iPSC-derived or adherent cells, see Suggestions for Expression in Adherent Cells section. For use in particularly difficult-to-transduce or low expressing cell types, see Alternative Spinoculation Protocol.

Table 1. Protocol applies to the following products (see product list for more detail):

Product	Description	Promoter
#C110N #C1130N - #C1150N	Host factors for SARS-CoV-2 viral entry	CMV
#K0002N - K0005N	GPCR Kinases	CMV
#R0001N - R0003N	RAMPs	CMV
#N0100N - #N0520N	Neurodegenerative Associated Proteins	CMV
#V0100N - #C0506N	Optogenetic Tool Kit	CMV / CAG / Synapsin
#X0250X	Gs Mutant	CMV
#X0000N	Empty BacMam	CMV
#Y0010N - Y0070N	Yamanaka Factors	CMV
#Z0100N - #Z2200N	GPCR Receptors in BacMam	CMV / CAG

Our goal is to make your workflow easy and reproducible.

We'd love to hear about your research.

Questions?
Call us, we can help!
+1 406-200-8321
info@montanamolecular.com

We also have a **Troubleshooting Guide** *at the end of this document*

BacMam Kit Materials and Storage

BacMam stocks should be stored at 4°C protected from light in the original package. **Avoid repeated freeze/thaw cycles.** We recommend re-testing BacMam stock after storing for more than 12 months. If your BacMam stock has been purified, use it within 30 days for best results. **Store sodium butyrate at 4°C.**

Table 2. Materials in Kit	Details	Storage
Product in BacMam ≅ 2 ×10^{10} VG/mL in ESF 921 Insect Culture Medium (Expression Systems, product #96-001-01)	CMV, CAG, or Synapsin driven expression, dependent on specific product.	4˚C
SB **sodium butyrate** (Sigma Aldrich product #B5887) 500 mM in H_2O	Sodium butyrate is added to the culture to maintain BacMam expression. Other HDAC inhibitors may work as well.	4˚C

Additional Materials Required (not included in kit)

1. Microplate coated with a cell attachment factor.
2. Cells and cell media of your choice.

Biosafety Considerations

The BacMam vector in this construct is a modified baculovirus, used for delivery to, and expression in, a wide variety of mammalian cell types including primary cultures.

BacMam is a modified baculovirus, *Autographa californica*, AcMNPV. The natural host of baculovirus is larvae of the order *Lepidoptera*. The BacMam vector in the kit is produced in the lab using Sf9 insect cells and is pseudotyped to infect mammalian cells. In mammalian cells, the baculovirus genome is silent, and it cannot replicate to produce new virus in mammalian cells. While it should be handled carefully, in a sterile environment, it is classified as a Biosafety Level 1 (BSL-1) reagent [3].

Other types of viruses are quantified in terms of plaque forming units (PFU) in cells from the natural host. Since BacMam is modified to produce expression in mammalian cells, we quantify the virus by measuring viral genes (VG) per milliliter (mL). Viral samples are prepared to release viral genomic DNA, then multiple dilutions of the preparation are run in qPCR using primers that are specific to the VSVG gene in the BacMam genome. Results are compared against a standard curve to generate an average titer for each viral stock. Check the label on the tube to find VG/mL for your stock.

This product is for research use only and is not for use or sale in human or animal diagnostic or therapeutic products.

Warranty

Materials are provided without warranty, express or implied. End user is responsible for making sure product use complies with applicable regulations. No right to resell products or any components of these products is conveyed.

Protocol for Expression

This protocol is optimized for use in HEK 293T cells, however, it can be adjusted for use with practically any cell type.

Take the time to optimize for your cell type and your particular conditions.

See our suggestions for Assays in Adherent Cell Types, Alternative Spinoculation Protocol.

Optimize Protein Expression

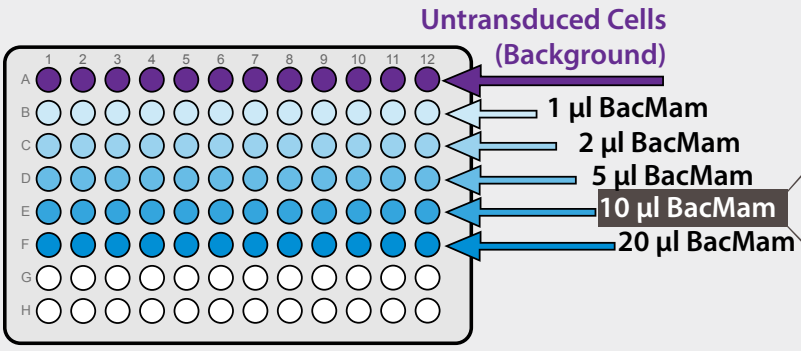
We recommend using a titration series with a known positive control to determine the best combination of signal to background and to confirm expression. The signal to be measured will be specific to your experimental needs.

__Tip:__ We offer untargeted fluorescent protein BacMam test kits that are a good way to determine BacMam transduction efficiency, evaluate promoter systems, and optimize expression in your cells. Questions: info@montanamolecular.com

Day 1

a. Set up your plate. Be sure to include control wells (untransduced cells) in order to calculate signal-to-background. **Refer to detailed protocol** Day 1 procedure **on page 5.**

b. Perform titration to determine optimal volume for your cells.

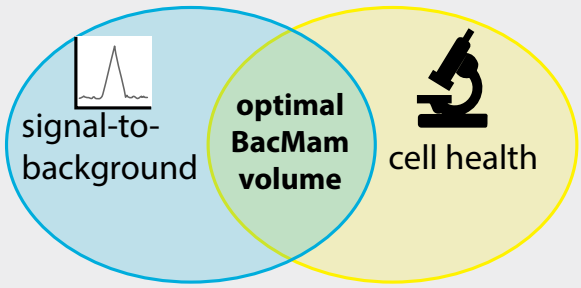


Transduction Reaction Per Well	
BacMam	10 µL
500 mM **SB** (sodium butyrate)	0.6 µL
complete media	39.4 µL
total volume per well	**50 µL**

Day 2

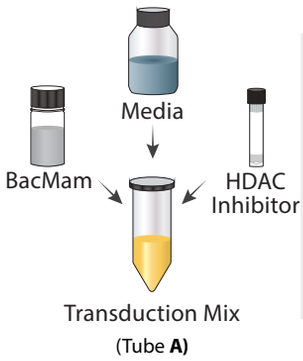
c. Measure signal to evaluate expression levels.

Determine optimal BacMam volume by checking your cells to compare cell health to signal-to-backround.



Day 1 – Transduce and Plate Cells for your Experiment

A. **Prepare Viral Transduction Mix (Tube A)**: For each transduction reaction (i.e. one well in a 96-well plate), prepare the transduction mix as detailed in table at right (using the optimal volume of sensor that was determined in your optimization experiment). Mix gently.



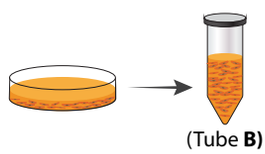
Transduction Reaction (Tube A)

Per Well (96-well plate)	
BacMam	Variable
500 mM (SB) sodium butyrate	0.6 µL
complete media	adjust to achieve 50 µL total volume per well

Tip: When preparing a master mix, scale up by 10-15% of the number of wells needed to avoid coming up short.

B. **Prepare Cells (Tube B):** Detach cells using standard trypsinization protocol. Resuspend cells in complete culture media and determine cell count.

C. **Prepare a dilution of cells at your desired concentration.** (100 µL of this cell resuspension will be required for a single well in a 96-well plate, so prepare enough of the dilution to seed the desired number of wells in the plate).

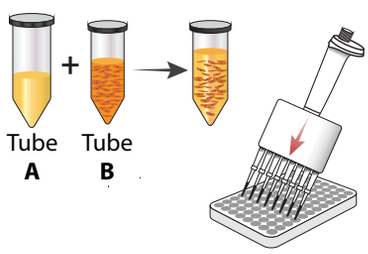


Cells (Tube B)

Per Well (96-well plate)	
cells	100 µL

Tip: 500,000 cells/mL works well for HEK293T cells. This will result in 50,000 cells/well in a 96 well plate. But remember, your optimal cell density is cell type dependent

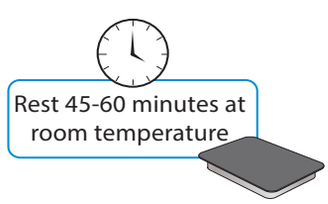
D. **Combine Transduction Mix and Cells**: Combine Tube A and Tube B (50 µL Tube A + 100 µL Tube B). Mix by pipetting up and down gently, and seed 150 µL per well on the 96-well plate.



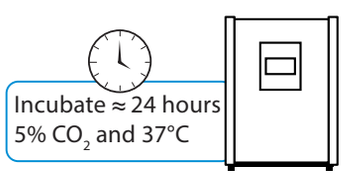
(Tube A) + (Tube B)
Transduction Reaction + Cells

Per Well (96-well plate)	
cell suspension	100 µL
transduction reaction	50 µL
total volume per well	**150 µL**

E. **Cover plate** to protect from light and let rest at room temperature for 45-60 minutes.



F. **Incubate ≈ 20-24 hours** under normal cell growth conditions (5% CO_2 and 37°C), protected from light.



Day 2 – Run Your Experiment

G. Twenty-four hours should suffice for expression of your gene product, however the cells can be used for several days depending on the health of the culture and the protein you choose to express. Experimental details and readouts will be unique to each product and project. Please reach out to us at info@montanamolecular.com if you have any questions or would like to discuss your experiments. If using these tools in combination with Montana Molecular's fluorescent sensors or other assays, additional protocols can be found on our site.

Suggestions for Expression in Adherent Cells

The protocol above is optimized for rapidly dividing immortalized cells. However, BacMam is compatible with screening primary cultures and iPSC-derived lines, where the cells are plated before transduction. Specific details of the protocol will vary by cell type, so it is important to take the time to titrate BacMam for optimal results. For expression in rare cell types, or specific cells in mixed cultures, Cre-dependent and specific promoter systems are available for many of our sensors.

Transduce Adherent Cells (Day 1)

Prepare Cells

a. For each transduction reaction (i.e. one well in a 96-well plate, containing 100 µL culture media per well), prepare a transduction solution by mixing an optimized volume of the BacMam with 0.6 µL of the 500 mM stock solution of (SB) sodium butyrate and cell culture media for a total volume of 50 µL. See page 4 for information on optimizing expression in your cells. Mix the solution gently. Cell culture media may be used in place of DPBS in the step above. **See the Optimization section** for more information.

b. Add the transduction reaction directly to the plated cells (no aspiration of cell media necessary). Gently rock the plate 4-5 times in each direction to mix throughout the well. Incubate the cells under normal growth conditions (5% CO_2 and 37°C), protected from light, for 20-24 hours.

c. Optional step (cell type dependent): After 4-8 hour incubation with the BacMam (6 hours is optimal), aspirate transduction solution and add 100 µL complete growth media with **SB** sodium butyrate at a concentration of 2 mM. Return cells to normal growth conditions for approximately 16-20 hours before measuring fluorescence as described above. If cells will not tolerate a full media exchange, partial media exchanges can be done.)

Measure Fluorescence

d. Reference Day 2 Detailed Procedure steps on page 8

Alternative Spinoculation Protocol

This alternative protocol may also be useful for particularly difficult-to-transduce or low expressing cell types:

a. Prepare transduction mix (detailed in table at right).

b. Detach cells using standard trypsinization protocol. Resuspend cells in complete culture media and perform cell count.

c. Prepare a dilution of cells at your desired concentration (22,500 cells/well in a 96-well plate is a good starting point, but will ultimately depend on the cell type being used). 50 µL of this cell resuspension will be required for a single well in a 96-well plate, so prepare enough of the dilution to seed the desired number of wells in the plate.

d. Combine the transduction mix with the cell suspension (**50 µL** transduction mix + **50 µL** cells). Mix gently, then seed **100 µL** of this mix per well on a 96-well plate.

e. Let cells sit at room temperature, protected from light, for 20 minutes.

f. **Spin the plate at 1,500 x g for 1.5-2 hours at room temperature.**

g. * We recommend sealing the plate with Breathe-Easy® (Cat. No. 70536-10) during this step to avoid contamination.

h. After spinning the plate, **remove the transduction mix** and replace with fresh media containing **0.6 µL sodium butyrate** (2 mM per well).

i. Return plate to normal growth conditions and incubate for 48 hours.

Alternative Transduction Mix	
BacMam	Variable
500 mM **SB** sodium butyrate	0.6 µL
1M HEPES (pH 7.4)	0.7 µL
cell culture media	(To 50 µL)
total volume per well	**50 µL**

Tip: *Titrate the sensor to determine optimal volume; see optimization page*

If the above protocol does not result in acceptable expression levels, we recommend using **System Biosciences' Spinoculation protocol**, which adds SBI's TransDux Max and MAX enhancer reagents to the transduction reaction. Please make the following adjustments to your transduction reaction:

Transduction Mix + Enhancer Reagents	
BacMam	Variable
TransDux	0.34 µL
Max Enhancer	17 µL
500 mM **SB** sodium butyrate)	0.6 µL
1M HEPES (pH 7.4)	0.7 µL
cell culture media	(To 50 µL)
total volume per well	**50 µL**

Optimization

Optimizing Expression for Your Cell Type

Optimization will vary depending upon which cell type you are using, which protein you are expressing, and what instrument you are using to make your measurements. In general a good start is to carefully titrate the virus to find optimal expression levels for your experiment. Typically a range of 1 to 20 µl of virus per well in a 96 well plate is a good starting point. Varying the cell density, concentration of sodium butyrate, or trying a new HDAC inhibitor (valproic acid or trichostatin A) may boost expression as well.

Please contact us if you would like to use the BacMam under the control of a specific promoter system. Weak promoters may limit expression and assay signal. To maintain strong expression in specific cell types, we recommend ordering a Cre-inducible, floxed construct.

Purified viral preparations, which can increase expression in particularly sensitive or difficult to transduce cell types, are available upon request.

References

1. Graham FL, Smiley J, Russell WC, Nairn R: Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J Gen Virol 1977, 36(1):59-74.
2. Kost T, Condreay J, Ames R, Rees S, Romanos M: Implementation of BacMam virus gene delivery technology in a drug discovery setting. Drug Discovery Today 2007, 12(9-10):396-403.
3. Pidre, M. L., Arrías, P. N., Amorós Morales, L. C., & Romanowski, V. (2022). The Magic Staff: A Comprehensive Overview of Baculovirus-Based Technologies Applied to Human and Animal Health. Viruses, 15(1). https://doi.org/10.3390/v15010080

Contact Us

If you have any questions about the protocols described here, or if you have ideas about how we can improve these tools, then we want to hear from you. Your feedback is extremely valuable. Please send an email to: info@montanamolecular.com or call us at **+1 406-200-8321** and we'll respond as quickly as we can.

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Troubleshooting Guide

Problem	Possible Cause	Solution
Low expression and/ or poor transduction efficiency	Suboptimal BacMam volume is being used.	Perform titration of the BacMam stock, testing a large range (i.e. 1-20 µL in 96-well plate format) to identify optimal volume. Too little can result in low expression, too much can cause cells to become sick.
	Transducing adherent cells.	Transduce cells while in suspension. If this isn't possible, try doing a media exchange on adherent cells after 4-6 hours, in addition to leaving the virus on overnight. See Suggestions for Expression in Adherent Cells.
	Suboptimal cell density; too few or too many cells added.	Transduce cells so that the cells will be around 75-80% confluent at the time of transduction. Also, when transducing cells in suspension, make sure that cells in the source flask are < 100% confluent (approximately 80% confluent is ideal).
	HDAC inhibitor was not added to the transduction mix, or the concentration was wrong.	Add HDAC inhibitor at the proper concentration: sodium butyrate - 2mM final concentration valproic acid - 5mM final concentration trichostatin A - 0.25µM final concentration * Perform a titration to determine optimal concentration for the cell type being used.
	HDAC inhibitor being used is not optimal for cell type.	Test other HDAC inhibitors (e.g. sodium butyrate, valproic acid, trichostatin A.)
	Cell type being used transduces poorly.	• After adding transduction mix to cells, let cells sit at room temperature for 30-40 min. before placing back in incubator (longer incubation times at room temperature may further improve transduction). • Perform media exchange after various incubation times with the transduction mix, in addition to leaving the virus on overnight. • Try high-titer, purified BacMam stock. • Validate assay in a different cell type (e.g. HEK 293T cells) • Transduce cells multiple times (e.g. on Day 1, and again on Day 2). • Incubate cells for 48 hours post transduction, before performing assay. • Consider using a different viral vector, such as lentivirus or AAV.
	Cell culture media is inhibiting transduction.	Remove media during transduction, preparing the transduction mix in PBS and adding to cells. Replace transduction mix with media after 2-4 hours.
	BacMam stock was not stored properly (i.e. not stored at 4°C, exposed to light for long periods, subjected to multiple freeze-thaw cycles), or the shelf life has been exceeded.	Follow guidelines for product storage. BacMam stocks are stable for at least 12 months when stored properly. After this time period, the stock should be re-evaluated and compared to previous experiments. Purified BacMam stocks should be used within 30 days for best results
	BacMam stock was not mixed adequately before transducing cells.	Mix BacMam stock thoroughly before transduction, especially after being stored for long periods.
	Promoter is not optimal for cell type being used	Identify promoters that work best in the cell type being used. If promoter is not on product list, consult Montana Molecular for custom production services.
	Cells are contaminated	Monitor cells for bacteria, fungi, etc