mNeonGreen BacMam

May 11, 2021

Product Info & Protocol

About mNeonGreen BacMam

The mNeonGreen BacMam kit and following protocol is useful to determine BacMam transduction efficiency and optimize expression in your cells of choice. You can also evaluate different promoter systems and use this information when ordering our fluorescent assay products.

For sensitive and/or difficult to transduce cell types, such as primary neurons, we offer purified, high-titer BacMam stock. Please **contact us** to discuss whether purified BacMam is the right product for you.

The following protocol is optimized in rapidly dividing, immortalized cell lines on a 96-well plate, and has been validated in live CHO, NIH 3T3, and HEK 293T cells [1]. For use in iPSC-derived or adherent cells, see **Suggestions for Expression in Adherent Cells** section. For use in CHO cells, see **Protocol Adjustments for CHO Cells** section.

This protocol applies to the products in Table 1:

Table 1. Relevant Products

Product	Description	Promoter	Recommended Use
#F0500G	mNeonGreen BacMam	CMV	Test expression efficiency in your cells
#F0505G	mNeonGreen BacMam	CAG	Test expression efficiency in your cells
#F0520G	Nuclear-Targeted mNeonGreen	CMV	Expresses Nuclear-targeted mNeon Green FP

BacMam Kit Materials and Storage

BacMam stocks should be stored at 4°C protected from light in the original package. Store **HDAC inhibitor at 4°C. 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.

Table 2. Materials in Kit	Details	Storage
mNeon in BacMam ≅ 2×10^{10} VG/mL in ESF 921 Insect Culture Medium (Expression Systems, product #96-001-01)	CMV or CAG driven expression, mNeon fluorescent protein in BacMam.	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

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*

Additional Materials Required (not included in kit)

1. Black, clear bottom microplate coated with a cell attachment factor. Greiner Cell Coat (#655946) is our preferred 96-well plate available from VWR.
2. Dulbecco's Phosphate Buffered Saline with Ca^{2+} and Mg^{2+} (PBS) available from VWR **[2]**.
3. Cells and cell media of your choice.

Biosafety Considerations

The BacMam vector carrying the fluorescent protein 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.

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 recommended for use or sale in human or animal diagnostic or therapeutic products.

Terms of Sale

All materials in this kit are provided without warranty, express or implied. 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. Use of materials is restricted to the intended purpose described in this protocol. Reverse engineering or modification of materials is not permitted. User agrees to accept these Terms of Sale before using materials.

Protocol for Use

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, Protocol Adjustments for CHO Cells..**

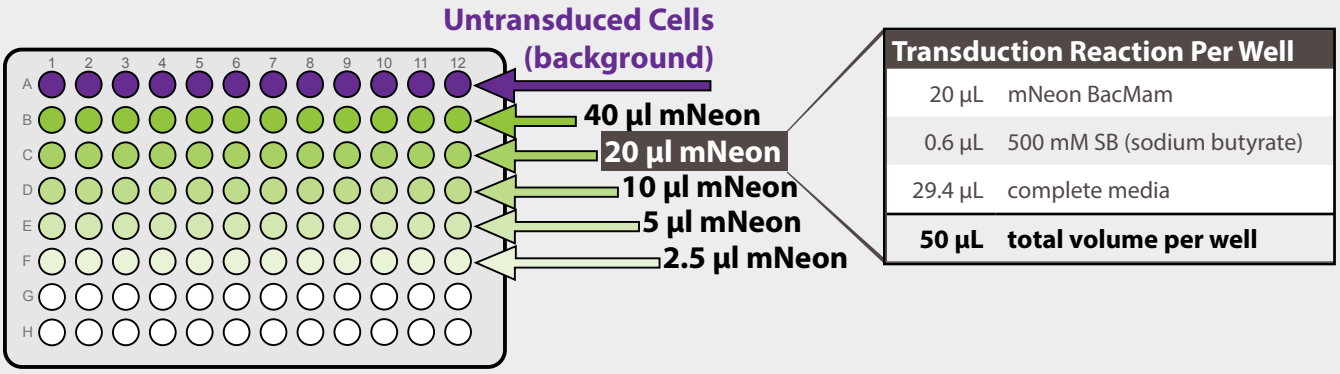
Optimize Fluorescent Protein Expression

We recommend using a titration series to determine optimal BacMam volume based on signal- above-background compared with cell health.

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.



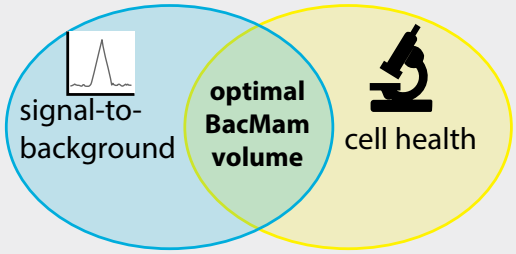
Transduction Reaction Per Well	
20 µL	mNeon BacMam
0.6 µL	500 mM SB (sodium butyrate)
29.4 µL	complete media
50 µL	**total volume per well**

Day 2

c. Measure fluorescence to evaluate expression levels.

d. **Refer to detailed protocol Day 2 procedure on page 8**

Check your cells for these 2 parameters to determine **optimal BacMam volume.**

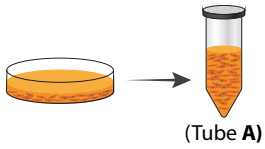


Day 1 – Detailed Procedure

Transduce and Plate Cells

Step 1)
Prepare Cells (Tube A)

a. Detach cells using standard trypsinization protocol. Resuspend cells in complete culture media and determine cell count.



b. 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. Let cells sit in hood and move on to preparation of the viral transduction reaction.

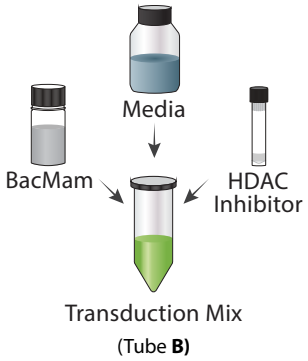
Cells (Tube A)

Per Well (96-well plate)	
100 µL	cells

***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*

Step 2)
Prepare Viral Transduction Reaction (Tube B)

c. For each transduction reaction (i.e. one well in a 96-well plate), prepare the transduction solution by mixing 20 µL of the mNeon BacMam stock with 0.6 µL of the 500 mM (SB) (stock solution of sodium butyrate), and 29.4 µL of the complete culture media for your cells, for a total volume of 50 µL. Mix gently. Following Step 3, the final concentration of sodium butyrate will be 2 mM.



Transduction Reaction (Tube B)

Per Well (96-well plate)	
20 µL	mNeonGreen
0.6 µL	500 mM (SB) sodium butyrate
29.4 µL	complete media

50 µL total volume

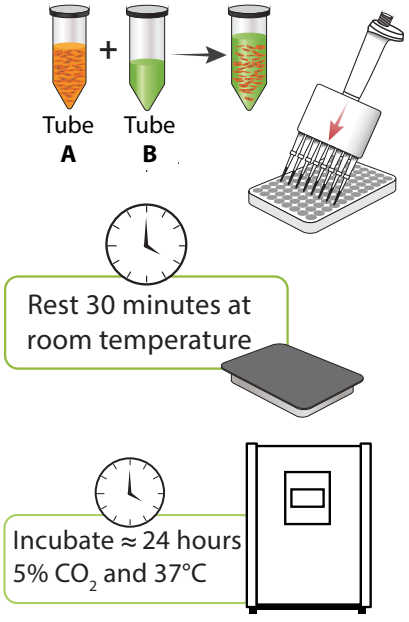
***Tip:** When preparing a master mix, scale up by 10-15% of the number of wells needed to avoid coming up short.*

Step 3)
Mix Cells and Transduction Mix

d. Mix Tube A and Tube B (100 µL tube A + 50 µL tube B). Mix gently and seed 150 µL of mix per well on the 96-well plate.

e. Cover plate to protect from light and let rest at room temperature for 30 minutes.

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



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

Per Well (96-well plate)	
100 µL	cell suspension
50 µL	transduction reaction

150 µL total volume per well

Protocol Adjustments for CHO Cells

For best results in CHO cells on a fluorescence plate reader, we recommend a modified transduction protocol. To boost expression, **replace sodium butyrate with valproic acid** in the transduction reaction and use the **following protocol modifications** to set up the experiment in 96-well format:

Tip: *For best results when using valproic acid, prepare 50-100 µL aliquots and store at -20°C.*

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

b. Prepare a dilution of cells at your desired concentration (we recommend 22,500 cells/well in a 96-well plate, as a starting point). **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. Let cells sit in hood and move on to preparation of the viral transduction reaction.

c. Prepare transduction reaction (detailed at right)

d. Mix the cells with the transduction reaction (**50 µL** cells + **50 µL** transduciton mix). 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.**

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

Transduction Reaction

Per Well (96-well plate)	
15 µL	mNeon BacMam
1.7 µL	300 mM valproic acid (5.1 mM in well)
0.7 µL	1M HEPES
32.6 µL	cell culture media
50 µL	**total volume**

g. After spinning the plate, **remove the transduction mix** and replace with fresh media containing **5 mM valproic acid** (100 µL-150 µL per well).

h. Return plate to normal growth conditions and incubate for 20-24 hours.

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 Reaction + Enhancer Reagents

Per Well (96-well plate)	
15 µL	mNeon BacMam
0.34 µL	TransDux
17 µL	Max Enhancer
1.7 µL	300 mM valproic acid (5.1 mM in well)
0.7 µL	1M HEPES
15.26 µL	cell culture media
50 µL	**total volume**

Suggestions for Expression in Adherent Cells

The protocol detailed on page 5 is optimized for rapidly dividing immortalized cells. However, mNeon 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)

Step 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 20 µL of the mNeon BacMam stock with 29.4 µL of culture media, and 0.6 µL of the 500 mM stock solution of (SB) sodium butyrate for a total volume of 50 µL. Mix the solution gently. mNeon expression and cell health can be controlled by titrating the virus, so it is worth taking the time to **optimize expression for your particular cell type** (see page 4). Cell culture media may be used in place of PBS in the step above. See the **Optimization section** on page 9 for more information.

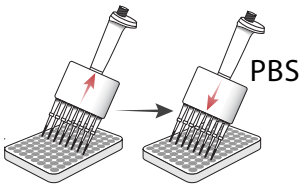
b. Add the transduction reaction directly to the plated cells (no aspiration of cell media necessary). Gently rock the plate 4-5 times in each direction to mix throughout the well. Incubate the cells under normal growth conditions (5% CO_2 and 37°C), protected from light, for 20-24 hours.

c. Optional step (cell type dependent): After 4-8 hour incubation with mNeon BacMam (6 hours is optimal), aspirate transduction solution and add 100 µL complete growth media with 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.

Step 2)
Measure Fluorescence

d. Reference **Day 2 Detailed Procedure** steps on page 8

Day 2 – Detailed Procedure

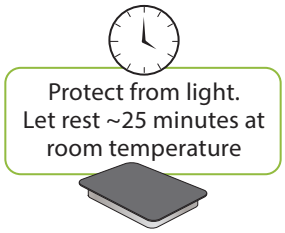


Measuring Fluorescence

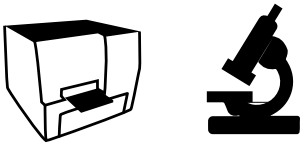
g. Prior to measuring fluorescence, replace culture media with PBS (1X, containing Ca^{2+} and Mg^{2+}). Wash gently so as not to dislodge cells. **Cover the cells and allow to rest at room temperature in PBS for ~25 minutes before measuring fluorescence so cells can adjust to the new environment.** Experiments are performed at room temperature.

h. Visually inspect cells on microscope to confirm cell health, mNeon expression, and transduction efficiency.

i. Measure signal-to-background. Transduced cells should be at least 5× brighter compared to untransduced cells.



*** Important:** Make sure that the final concentration of any drug solvents in the well (e.g. DMSO or Ethanol) does not exceed 1.0%.



Fluorescence Detection

Our BacMam products are compatible with automated fluorescent plate readers and imaging systems.

Our customers have reported good results on:

- Hamamatsu FDSS®
- Molecular Devices FLIPR®
- Molecular Devices Flexstation®
- Perkin Elmer Enspire®

We have validated on:

- Biotek Synergy MX™
- Biotek Cytation™
- BMG CLARIOstar®
- Epifluorescence microscopes

Fluorescence Properties

mNeonGreen BacMam expresses the very bright, mNeonGreen fluorescent protein [5]. While the peak excitation and emission wavelengths are 506 nm and 517 nm, respectively, a range of 485-505 nm (excitation) and 515-535 nm (emission) may be used if your instrument does not allow measurement at the peak ex/em. For example, on the BioTek Synergy MX™, the preferred ex/em is 488/525. If using filters, we recommend Chroma's Catalog set #49003 for optimal results.

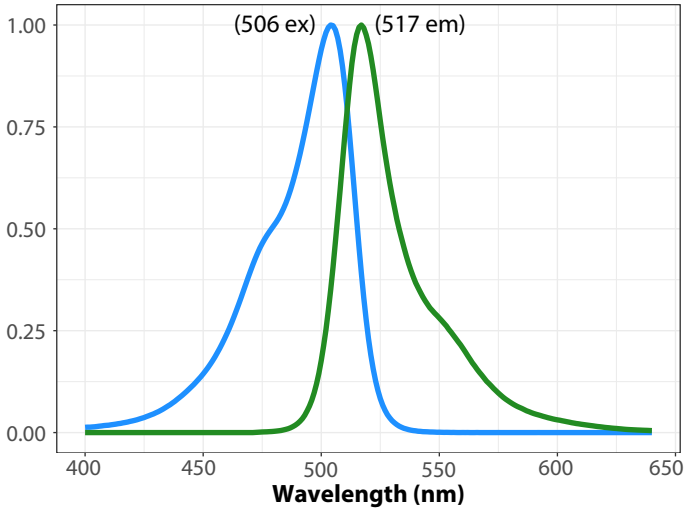


Figure 1. Absorption and emission properties of the mNeonGreen fluorescent protein plotted as a function of wavelength.

Optimization

Optimizing Fluorescence

Twenty-four hours after transduction, check your cells for fluorescence. Wells that were transduced with the mNeonGreen BacMam should be 5-10 times brighter than control wells that were not treated with the BacMam.

HDAC inhibitors may be used to maintain expression. While BacMam transduction alone will result in protein expression, sodium butyrate or another HDAC inhibitor, such as valproic acid (VPA) or trichostatin A (TSA), will generate higher levels of expression and will maintain this level of expression. If cells look unhealthy, use lower concentrations or no HDAC inhibitor.

The type of cell culture media used in your experiment can affect the transduction efficiency of BacMam. Our assays have been validated in EMEM, DMEM, McCoy's 5A, and F12K culture media.

Optimizing Expression for Your Cell Type

To determine optimal conditions for your cell type, prepare a dilution series of transduction reactions by varying the amount of BacMam. For example, a range of 2.5 µL to 40 µL is a good starting range in a 96-well plate..

Varying the cell density, concentration of sodium butyrate, or trying a new HDAC inhibitor (VPA or TSA) 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 be limited to detection on imaging systems. 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.

Troubleshooting Guide

Problem	Possible Cause	Solution
1. 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. 2.5-40 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 valproic acid - 5mM trichostatin A - 0.25µM * 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.

Problem	Possible Cause	Solution
(continued) **Low expression and/or poor transduction efficiency**	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.
2. Low fluorescence signal on microscope/plate reader.	Low expression, low transduction efficiency.	See solutions for **Problem 1**.
	Excitation/emission settings are not optimal for mNeon.	Refer to protocol for the **fluorescence spectra** of the mNeon. Make sure that filter sets or monochromators are aligned with the peak excitation and emission wavelengths.
	Cells are in cell culture media, and the media is producing a large fluorescent signal (autofluorescence).	Exchange media so that cells are in PBS at the time of experiment. If cell culture media must be used, try using FluoroBrite media.
	Wrong microplate type is being used.	Use black, clear-bottom microplates with low autofluorescence.
	Exposure time or gain setting on instrument is suboptimal.	Test different exposure and gain settings, monitoring how the signal-to-background and noise in the measurement changes. Too high can result in saturation and/or photobleaching; too low can result in noisy data.
	Cells were dislodged during media exchange/plate washing.	Make sure that media exchange or plate washing is done gently and does not dislodge cells. **Confirm with visual inspection on a microscope**.
3. Signal-to-background is low (i.e. cells/wells with mNeon are not much brighter than control cells/wells without the fluorescent protein.	Low expression, low transduction efficiency.	See solutions for **Problem 1**.
	Excitation/emission settings are not optimal.	Refer to protocol for the **fluorescence spectra**. Make sure that filter sets or monochromators are aligned with the peak excitation and emission wavelength.
	Exposure time or gain setting on instrument is suboptimal.	Test different exposure and gain settings, monitoring how the signal-to-background and noise in the measurement changes. Too high can result in saturation and/or photobleaching; too low can result in noisy data.
	Media exchange was not performed before running the assay; cells are in media rather than PBS. Cell culture media being used has high autofluorescence.	Perform media exchange so that cells are in PBS at the time of experiment. If cell culture media must be used, try using FluoroBrite media.
	Cells were dislodged during media exchange/plate washing.	Make sure that media exchange or plate washing is done gently and does not dislodge cells. **Confirm with visual inspection on a microscope**.

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. Dulbecco R and Vogt M: Plaque formation and isolation of pure lines with poliomyelitis viruses. The Journal of experimental medicine 1954.
3. Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC: Green fluorescent protein as a marker for gene expression. Science 1994.
4. 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.
5. Shaner, N.C., Lambert, G.G., Chammas, A., Ni, Y., Cranfill, P.J., Baird, M.A., Sell, B.R., Allen, J.R., Day, R.N., Israelsson, M., Davidson, M.W., & Wang, J. (2013) "A bright monomeric green fluorescent protein derived from Branchiostoma lanceolatum." Nature Methods, May;10(5):407-9. doi: 10.1038/nmeth.2413.

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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