Montana Molecular
Fluorescent Biosensors for Live Cell Discovery

# Red R-GECO BacMam Assay

Product Numbers: #U0600R

## Overview

This document is a guide for expressing robust, red fluorescent protein-based R-GECO sensors in mammalian cells. The protocol is optimized for HEK 293 cells [1] in a 96-well plate and is appropriate for live-cell imaging and for screening on automated fluorescence plate readers.

## Materials included

- R-GECO sensor in BacMam under the control of a CMV promoter.

  Baculovirus stock should be stored at 4°C and protected from light. For long-term storage (more than 6 months), store aliquots of the virus at -80°C. Avoid repeated freeze/thaw cycles.

- Sodium Butyrate (Sigma Aldrich product number B5887) 500 mM.

  Sodium Butyrate is added to the culture to maintain BacMam expression. Other HDAC inhibitors may work as well.

## Additional materials

- Greiner CellCoat (#655946) is our preferred plate for this assay.
- Dulbecco's Phosphate Buffered Saline [2].

## Sample Protocol Optimized for HEK 293 cells.

### Day 1

- Seed HEK 293 cells at a density of 30,000 cells/well in 100 ul complete growth medium. Allow cells to grow overnight under normal growth conditions (5% CO2 and 37°C).

### Day 2

- Prepare a 500 mM stock solution of sodium butyrate in sterile water.
- For each transduction reaction (i.e. one well in a 96-well plate), prepare the transduction solution by mixing 25 ul of the baculovirus stock with 23.5 ul of DPBS and 1.5 ul of the 500 mM stock solution of sodium butyrate for a total volume of 50 ul.
- Mix the solution gently, then add directly to the well (no aspiration of cell medium necessary). Gently rock the plate 4-5 times in each direction to mix throughout the well.

Incubate the cells under normal growth conditions, protected from light, for 6 hours (5% CO2 and 37°C).

- Aspirate transduction solution and add 100 ul complete growth medium with sodium butyrate at a concentration of 1-2 mM. Return cells to normal growth conditions for approximately 36-48 hrs.

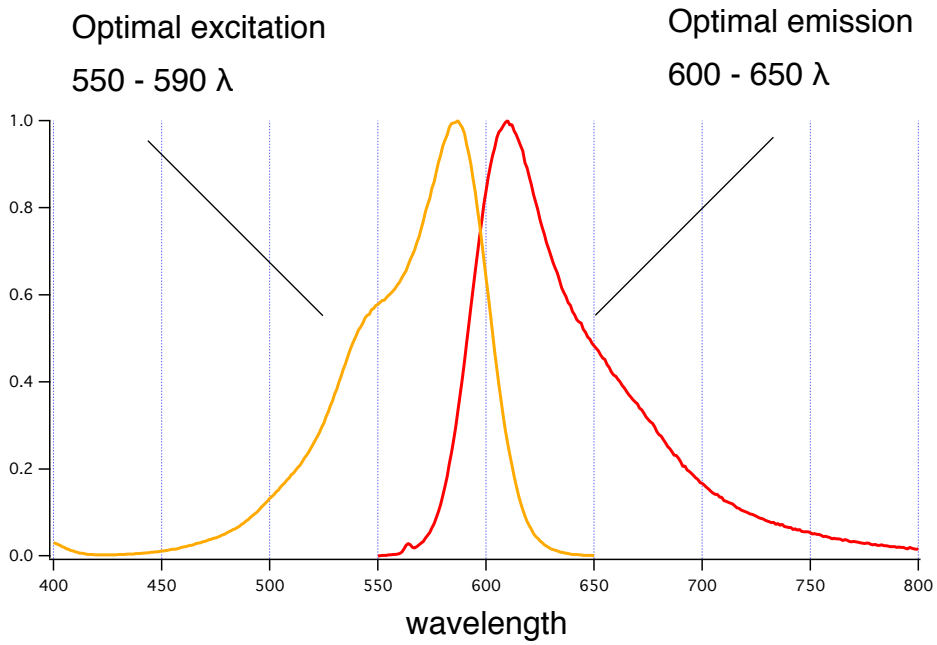
### Day 4

- Cells are now ready for assay. Prior to imaging or scanning on a fluorescence plate reader, replace culture media with DPBS. Experiments are performed at 25°C. When monitoring the red fluorescence emitted by the sensor, a change in red fluorescence intensity will be observed after addition of compounds that increase levels of $Ca^{2+}$ in the cell.

## Detection

### Fluorescence Properties

This sensor is constructed using a red fluorescent protein. The optimal excitation wavelength is 590 nm, but the absorption band of this protein is quite broad, so broad bandpass filters that pass 550 to 590 nm light can be used quite effectively. On the emission side, the red light spans 600 to 700 nm, so broad band pass emission filters can also help to collect much of the emission.



### Timing

This is a live cell assay, and unlike many assays that measure accumulation an analyte over time, this sensor detects, real time, the $Ca^{2+}$ level in the living cell. Changes in $Ca^{2+}$ can occur quite rapidly, so the application of drug and resulting changes should be captured as quickly as possible. The best possible experimental setup involves capturing the fluorescence from a well of cells first, before the addition of any compound, and then again a second or two after the compound addition. Sampling the fluorescence at intervals of 1-2 seconds provides a good measurement of the response. The maximal response is reached at ~ 3 seconds after the addition of the agonist and in HEK293 cells, returns to baseline after 10-12 seconds.

### Validated detection systems.

This red fluorescent sensor has been validated on a variety of epifluorescence microscopes with lenses ranging from 20 X, 0.9 N.A. to 63X, 1.4 N.A. Before attempting to deploy this sensor in automated readers, we recommend you optimize the assay on a microscope.

## Factors Influencing Assay Performance

### How we measure the infectivity of the viral stock.

Oftentimes, virus infectivity is measured in terms of plaque forming units in the natural host. There can be significant differences between infection of the Sf9 insect cell host for baculovirus and the efficiency of the virus to transduce any given mammalian cell. Therefore, we measure transducing units that are capable of producing expression in mammalian cells. In each batch of baculovirus we produce, we transduce HEK 293 cells with serial dilutions of the virus and count the fluorescent cells at dilutions sufficient to produce individual transduction events. For the upward series of sensors, we stimulate signaling by addition of an agonist and then count the cells.

### Expression levels of the sensor.

To optimize the assay for your particular cell type, it is important to titrate the amount of virus used in the transduction. Too little virus will produce variable results particularly if the sensor expression levels are low and difficult to detect on your instrument.

### Level of receptor expression.

The magnitude of the sensor response can be affected by the level of GPCR expression in your cells. We have found that low levels of receptor expression often produce the largest signals, while high levels of receptor expression often produce smaller responses. This is consistent with the observation that over expression of some GPCRs can change the levels of second messengers due to low levels of spontaneous activity.

# Trouble Shooting

This is a robust, easy to use assay. If you encounter problems, there are several steps that you can consider when troubleshooting.

### Are the cells fluorescent?

This virus drives expression in mammalian cells because a CMV promoter has been positioned in front of the sensor coding region. CMV is an effective promoter in many cell lines, but not all. When you add the virus, are you seeing bright fluorescent cells in a microscope? Do you know that the CMV promoter works in your cell line? Twenty four hours after transduction with the R-GECO virus, you should see red fluorescent cells in a typical fluorescent microscope, or the transduced wells in a 96 well plate should be significantly more fluorescent than un transduced cells in wells on the same plate.

HDAC inhibitors are important to expression of the sensors. While baculovirus transduction alone will initially generate low levels of sensor expression, it is important to include sodium butyrate to generate optimal levels of sensor expression and maintain this level of expression [4].

### Contact us!

After following the suggested protocol and trouble shooting steps, if you still have questions or feedback for us, please let us know. We strive to respond to emails sent to info@montanamolecular.com within 24 hours and usually respond much sooner.

# Bibliography


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, 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.