Montana Molecular
Fluorescent Biosensors for Live Cell Discovery

Green cADDis™ cAMP Assays

February 12, 2024

Product Info & Protocol

US Patent 11,366,114 B2
European Patent Number: EP3065754B1

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About these Assays

Cyclic AMP (cAMP) is an essential second messenger for many cellular processes. The messages carried by cAMP are tightly regulated within cells. The cADDis assays detect changes in cAMP in living cells. We also have cADDis assays that can be targeted to specific populations of cells in mixed cultures or to subcellular compartments; contact us to discuss these additional tools. The cADDis assays for cAMP can be combined with different colored sensors to measure multiple signals simultaneously.

Depending upon the sensor in your kit (Downward or Upward), fluorescence either decreases or increases. For example, #D0200G Green Down cADDis **decreases in fluorescence** when cAMP levels **increase** in the cell. Both cADDis sensor versions are bright, robust, and easy to detect on fluorescence plate readers (Fig. 6). For sensitive and/or difficult to transduce cell types, such as primary cultures, we offer purified, high-titer BacMam stock. Please contact us to discuss whether purified BacMam is the right product for you.

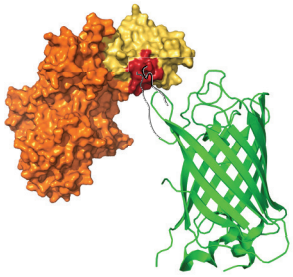


Figure 1. cADDis sensor is comprised of a circularly permuted mNeonGreen fluorescent protein fused to the hinge region of EPAC-2. EPAC-2 hinge domain shown in red, regulatory doman in yellow, catalytic domain in orange.

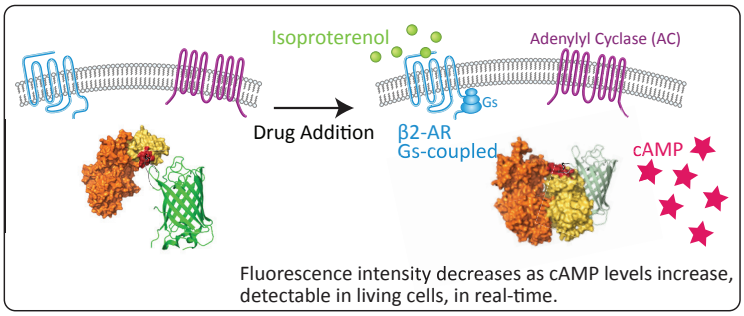


Figure 2A. Downward cADDis sensor function

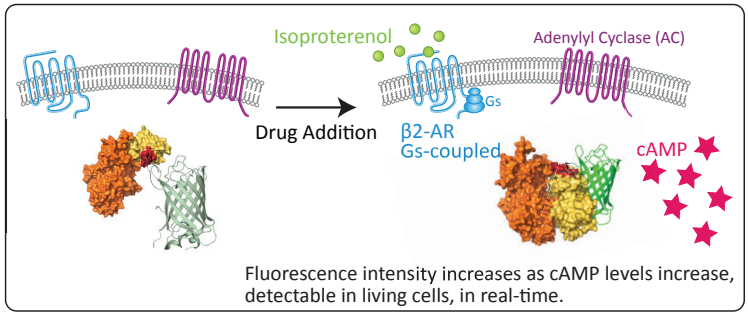


Figure 2B. Upward cADDis sensor function

The cADDis Assay protocol is optimized for measuring cAMP responses in rapidly dividing, immortalized cell lines on a 96-well plate, and has been validated in live HEK 293T cells [1]. cADDis users have published results in a wide variety of cell types. This assay is very robust and can be used for live-cell imaging or for screening on automated fluorescence plate readers. For use in iPSC-derived or adherent cells, see Suggestions for Assays in Adherent Cells section. For use in CHO cells or other cell types, see Alternative Spinoculation Protocol section.

The protocol steps are simple and will be performed over two consecutive days (Fig. 3). Please remember to optimize the assay for your conditions before performing your first experiment.

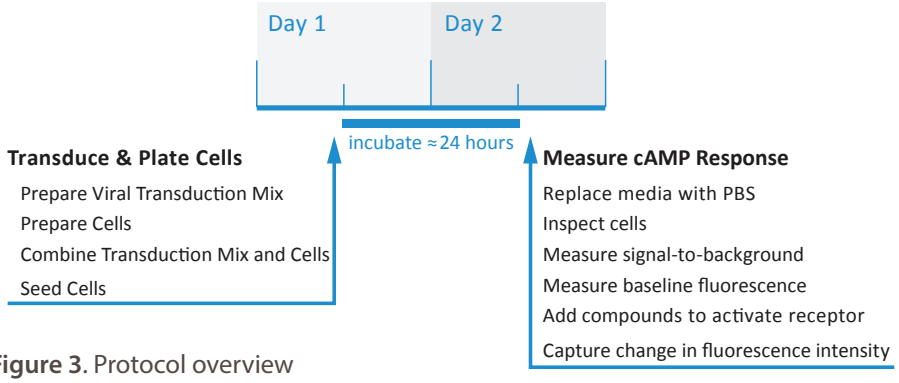


Figure 3. Protocol overview

Table 1. cADDis cAMP Assay Kits

Product	Description	Promoter	Recommended Use
#D0200G	Green Down cADDis cAMP	CMV	Fluorescence imaging and plate reader assay (Z' > 0.9)
#D0205G	Green Down cADDis cAMP	CAG	Fluorescence imaging and plate reader assay
#U0200G	Green Up cADDis cAMP	CMV	Fluorescence imaging and plate reader assay (Z' > 0.85)
#U0205G	Green Up cADDis cAMP	CAG	Fluorescence imaging and plate reader assay

Assay Kit Materials and Storage

BacMam stocks should be stored at 4°C protected from light in the original package. **Store control agonist at -20°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. **Store sodium butyrate at 4°C.**

Table 2. Materials in Kit	Details	Storage
cADDis cAMP sensor BacMam ≅ 2×10^{10} VG/mL in ESF 921 Insect Culture Medium (Expression Systems, product #96-001-01)	Green fluorescent sensor that changes in fluorescence intensity in response to increases in cAMP. VG/mL is the number of viral genes per milliliter (see Biosafety Considerations section).	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
Rs **β2 adrenergic receptor BacMam** in ESF 921 Insect Culture Medium (Expression Systems, product #96-001-01)	A Gs-coupled receptor provided as a positive control for the purpose of assay optimization. Contains a separate red fluorescent protein that is targeted to the nucleus. (The control receptor included in CAG-promoter kits does not have a separate fluorescent protein.)	4°C
As **isoproterenol 10 mM** (Sigma Aldrich Product Number I2760) in 10 mM HCl	Isoproterenol can be used to stimulate Gs signaling through the positive control, the β2 adrenergic receptor.	-20°C

Additional Materials Required (not included in kit)

1. Black, clear bottom microplate coated with a cell attachment factor. We recommend the following plates; 96-well Greiner Bio-One (#655946), 96-well Thermo Fisher Scientific (#152037), 384-well Greiner Bio-One: (#781946), 384-well Corning: (#354663).
2. Dulbecco's Phosphate Buffered Saline 1X with Ca^{2+} and Mg^{2+} (10X solution from Gibco #14080055) [2].
3. Cells and cell media. We recommend media with low autofluorescence such as EMEM, McCoy's 5A, and F12K culture media.

Biosafety Considerations

The BacMam vector carrying the fluorescent biosensor in these assays 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 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. While it should be handled carefully, in a sterile environment, it is classified as a Biosafety Level 1 (BSL-1) reagent [9].

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.

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 the assay for your cell type and your particular conditions.
See our suggestions for Assays in Adherent Cell Types, Scaling for 384-well Plates, and Alternative Spinoculation Protocol.

Before your first experiment: Optimize Your Assay by Titrating the Sensor

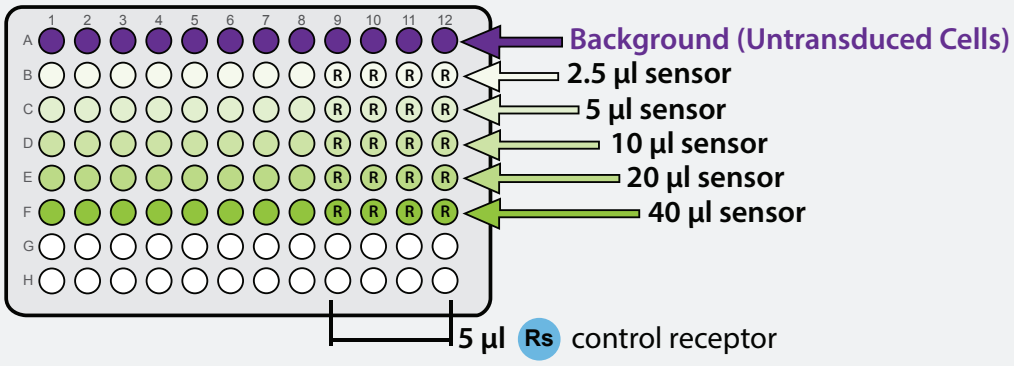
We recommend using a titration series to determine the best combination of signal above background, cell health, and sensor expression. Ideally, the signal in each well before drug addition should be at least 5 times above background.

Transduce and Plate Cells for Optimization

A. Set up your plate. Be sure to include control wells (untransduced cells) in order to calculate signal-to-background. (For details on preparing cells and transduction mix, refer to Day 1 procedure on page 5).

B. Perform titration to determine optimal sensor volume for your cells, as detailed in plate diagram and tables below.

C. For each sensor volume, include a subset of wells that includes 5 µl of **Rs** control receptor included in your kit. These wells will be used for your positive control.



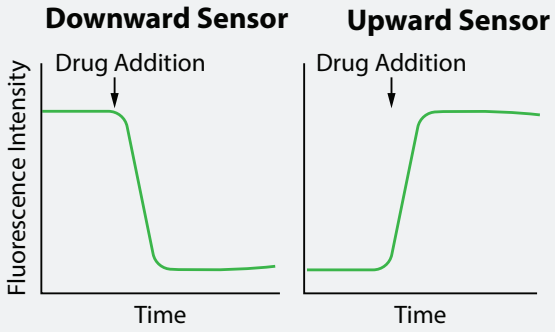
Wells A1-A12 Background (with Untransduced Cells)	
500 mM **SB** (sodium butyrate)	0.6 µL
complete media	49.4 µL
total volume per well	**50 µL**

Wells B1-B8 Transduction Mix	
cADDis cAMP Sensor	2.5 µL
500 mM **SB** (sodium butyrate)	0.6 µL
complete media	46.9 µL
total volume per well	**50 µL**

Wells B9-B12 Transduction Mix	
cADDis cAMP Sensor	2.5 µL
500 mM **SB** (sodium butyrate)	0.6 µL
Rs (β2 adrenergic control receptor))	5 µL
complete media	41.9 µL
total volume per well	**50 µL**

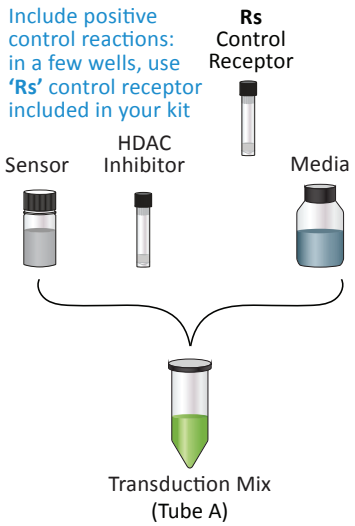
Measure Parameters to Determine Optimal Conditions

D. Determine optimal sensor volume by analyzing fluorescence above background, cell health, and response to drug. (For details on measuring fluorescence, refer to Day 2 procedure.)



Day 1 – Transduce and Plate Cells for your Experiment

E. **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.



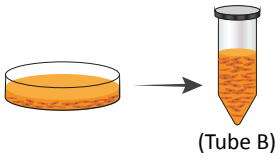
Transduction Mix (Tube A)	
cADDis cAMP Sensor	Variable
SB 500 mM sodium butyrate	0.6 µL
Rs control receptor (if using)	5 µL
complete media	(To 50 µL)
total volume per well	**50 µL**

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

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

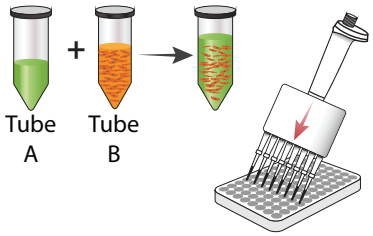
Cells (Tube B)	
cells per well	100 µL

G. **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).



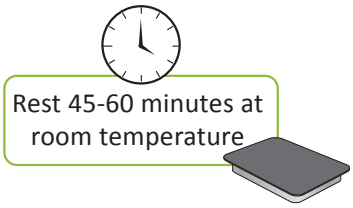
Transduction Mix + Cells (Tube A) + (Tube B)	
transduction mix	50 µL
cell suspension	100 µL
total volume per well	**150 µL**

H. **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.

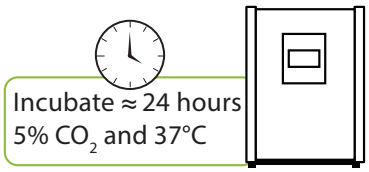


Tip: *90% cell confluence after 24 hours is ideal. For HEK 293T cells 50,000 cells per well works well but plating density is cell type dependent*

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



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



Suggestions for Assays in Adherent Cells

This protocol is optimized for rapidly dividing immortalized cells.

However, cADDis biosensor assays can be used with primary cultures of cells as well as iPSC-derived cells. In the case of non-dividing, or differentiated cells, the transduction should be done with adherent cells, and the media should be exchanged the following day. 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.

***When working with adherent cell cultures, add an extra day to seed the cells prior to transduction.**

Adherent Cells Day 1:

- Seed the cells the day before you add transduction mix, incubate 24 hours

Adherent Cells Day 2:

- Perform transduction as directed in step E, but add the transduction reaction directly to the plated cells (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 (5% CO_2 and 37°C), protected from light, for 20-24 hours.
- Optional step (cell type dependent): After 4-8 hour incubation with sensor BacMam (6 hours is optimal), very gently aspirate transduction solution (we recommend using a plate washer). Add 100 µL complete growth media with sodium butyrate at a concentration of 2 mM. If cells will not tolerate a full media exchange, partial media exchanges can be done.

Adherent Cells Day 3:

- Measure Fluorescence as as detailed in Day 2 procedure.

Scaling for 384 Wells (1 plate)

To set up the assay in a 384-well plate, follow all of the protocol steps, adjusting reagent volumes as follows:

Transduction Mix (Tube A) (384-well plate)	
cADDis cAMP Sensor	2 µL
500 mM **SB** sodium butyrate	0.1 µL
Rs control receptor	1 µL
complete media	9.4 µL
total volume per well	**12.5 µL**

Cells (Tube B) (384-well plate)	
cells per well	12.5 µL

12.5 µL of the cell resuspension will be required for a single well in a 384-well plate. A plating density of 7,500 cells per well is a good starting point, so prepare the cell suspension at 600,000 cells/mL. Depending on the cell type and plate type, 5,000-15,000 cells per well may be optimal.

Transduction Mix + Cells (Tube A) + (Tube B) (384-well plate)	
transduction mix	12.5 µL
cell suspension	12.5 µL
total volume per well	**25 µL**

Tip: *When scaling for 384-well plates, the volume of the cell suspension per well can be increased to 50 µL to improve cell health. Make sure that the well is still receiving 7,500 cells and that you increase the volume of sodium butryate to 0.2 µL per well.*

Alternative Spinoculation Protocol for Other Cell Types

For best results in CHO cells on a fluorescence plate reader, we recommend a modified transduction protocol. This alternative protocol may also be useful for other cell types.

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

1. Prepare transduction mix (detailed in table at right).
2. Detach cells using standard trypsinization protocol. Resuspend cells in complete culture media and perform cell count.
3. 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.
4. 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.
5. Let cells sit at room temperature, protected from light, for 20 minutes.
6. **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.
7. After spinning the plate, **remove the transduction mix** and replace with fresh media containing **5 mM valproic acid** (100 µL-150 µL per well).
8. Return plate to normal growth conditions and incubate for 20-24 hours.

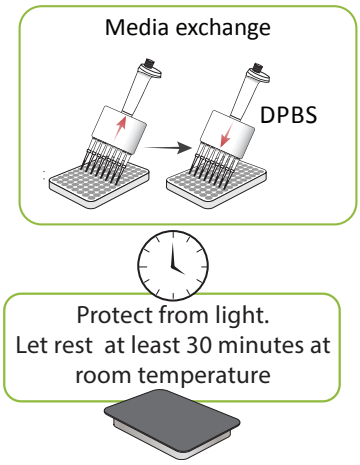
Transduction Mix for CHO Spinoculation	
cADDis cAMP Sensor	Variable
Rs control receptor	5 µL
300 mM valproic acid (5.1 mM in well)	1.7 µL
1M HEPES (pH 7.4)	0.7 µL
cell culture media	(To 50 µL)
total volume per well	**50 µL**

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 mix. Please make the following adjustments to your transduction mix:

Transduction Mix + Enhancer Reagents	
cADDis cAMP Sensor	Variable
Rs control receptor	5 µL
TransDux	0.34 µL
Max Enhancer	17 µL
300 mM valproic acid (5.1 mM in well)	1.7 µL
1M HEPES (pH 7.4)	0.7 µL
cell culture media	(To 50 µL)
total volume per well	**50 µL**

Day 2– Measure cAMP Response

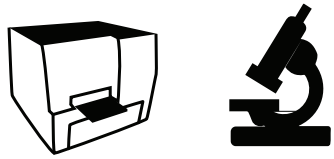


K. Prior to measuring fluorescence, replace culture media with DPBS (1X, containing Ca^{2+} and Mg^{2+}). Wash gently 4-5 times, careful not to dislodge cells. We recommend using a plate washer. (We recommend the BioTek 405 TS.)

L. Cover the cells. Allow to equilibrate at room temperature at least 30 mintues before measuring fluorescence. Experiments can be performed at room temperature

M. Visually inspect cells on microscope to confirm cell health. Transduced cells should be at least 5× brighter compared to untransduced cells. (If you have high background fluorescence, gently wash cell culture media again.)

Measure Fluorescence on Plate Reader or Imaging System

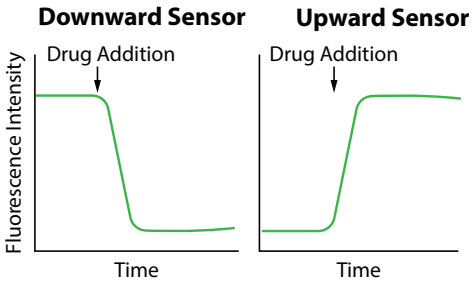


N. Measure fluorescence in transduced and untransduced cells. Transduced cells should be at least 5× brighter compared to untransduced cells.

O. Acquire 10-20 baseline fluorescence reads before adding compounds.

P. Activate the receptor with agonist to affect levels of cAMP. Resume measurement immediately after adding drug. Measure continuously for a minimum of 30 minutes, (and up to 5 hours at 15-60 second intervals on a plate reader). A change in fluorescence intensity under standard GFP excitation and emission will indicate a change in cAMP levels. For wells transduced with the β2 adrenergic control receptor, add 10 µM **As** (isoproterenol, final concentration in well) to activate the receptor. The optimal dose of agonist may need to be determined for a given cell line.

* **Important:** Make sure that the final concentration of any drug solvents in the well (e.g. DMSO) does not exceed 1%. The ideal concentration for DMSO is 0.1%.



Tip: *Add agonists at a concentration of 3-4× (in a volume of 50 µL DPBS) to wells containing 100-150 µL DPBS. This will result in a 1:3 or 1:4 dilution, and allow for adequate diffusion.*

Tip: *Always test the addition of the vehicle alone (i.e. DPBS without drug).*

Fluorescence Detection

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

We have validated on:

- Agilent (BioTek) Synergy
- Agilent (BioTek) Cytation™
- BMG CLARIOstar®
- Agilent (BioTek) Lionheart
- Agilent (BioTek) Neo
- Hamamatsu FDSS®
- Epifluorescence microscopes

Our customers have reported good results on:

- Molecular Devices FLIPR®
- Molecular Devices Flexstation®
- Perkin Elmer Enspire®
- Perkin Elmer Opera Phenix ®

Tip: *Below are the specific settings recommended for use with our assays for instruments we use at Montana Molecular. To determine the best settings for fluorescence detection on your instrument, please consult the manufacturer.*

Table 3. CLARIOstar® Recommendations

Instrument Settings	
Detection Mode:	FI (Bottom)
Detection Method:	Plate Mode, Kinetic
Scan Mode:	Orbital Averaging
Scan Diameter (mm):	4
Gain/Focal Height:	Adjusted prior to test
Optical Settings	
Excitation:	F 482-16
Dichroic:	LP 504
Emission:	F 530-40

Table 4. Neo & MX™ Recommendations

Instrument Settings	
Detection Method:	Fluorescence Intensity
Read Type:	Endpoint/Kinetic
Optics:	Monochromators
Excitation:	485 nm
Emission:	528 nm
Bandwidth:	20 nm (for both ex and em)
Optics Position:	Bottom
Gain:	100

Table 5. Cytation™ & Lionheart Recommendations

Image Preprocessing	
Image Set:	GFP
Background:	Dark
Rolling Ball Diameter:	Automatic
Image Smoothing:	0 cycles
Cellular Analysis Parameters	
Channel:	Tsf [GFP]
Threshold:	7,000
Background:	Dark
Split Touching Objects:	Checked
Fill Holes in Mask:	Checked
Minimum Object Size:	5 µm
Maximum Object Size:	1,000 µm
Include Primary Edge Objects:	Checked
Analyze Entire Image:	Checked
Advanced Detection Options	
Rolling Ball Diameter:	Automatic
Image Smoothing Strength:	1 cycle of 3×3 average filter
Evaluate Background On:	5%
Primary Mask:	Use threshold mask

Fluorescence Properties

Green cADDis is constructed with the very bright, mNeonGreen fluorescent protein [6]. 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.

Tip: *We offer mNeon BacMam Kits (products #F0500G and #F0505G). These test kits are a good way to determine BacMam transduction efficiency, evaluate promoter systems, and optimize expression in your cells of choice.*

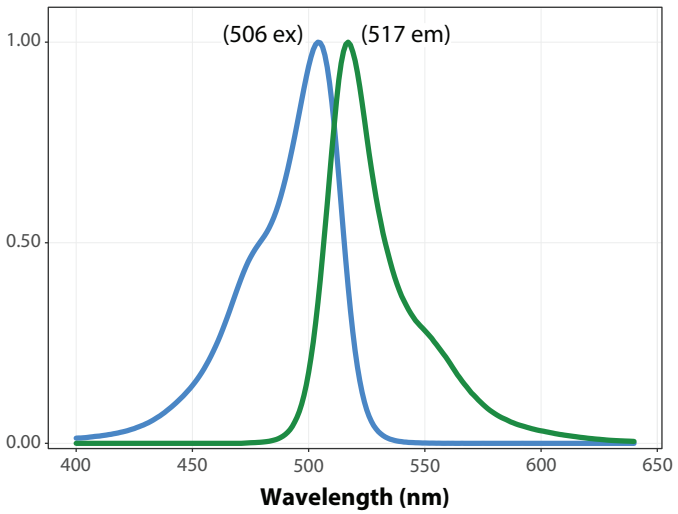


Figure 4. Absorption and emission properties of the mNeonGreen fluorescent protein plotted as a function of wavelength.

Timing

Unlike many assays that measure accumulation of cAMP in cell lysates, the cADDis assay measures cAMP in living cells, in real time. For best results, be sure to capture changes in cAMP during the peak response. In Figure 5, fluorescence was captured from cells before the addition of the drug and then sampled at regular intervals. The maximal response is reached at 5-10 minutes after the addition of the drug.

Data Analysis

Check out our helpful reference document "Biosensor Analysis Overview"

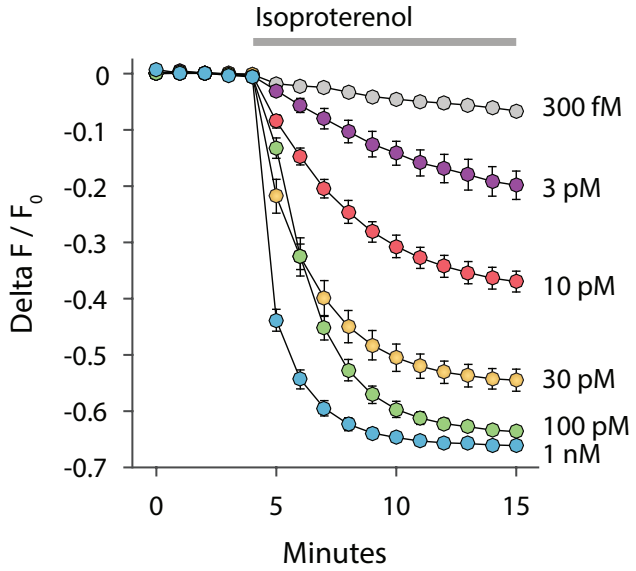


Figure 5. HEK 293T cells transduced with 20 µL of BacMam carrying Green Down cADDis cAMP biosensor, activated with isoproterenol. The graph shows the expected timing and duration of cAMP when using the positive controls provided in your kit.

Assay Optimization

Optimizing Fluorescence

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

HDAC inhibitors may be used to maintain expression of the sensors. While BacMam transduction alone will result in sensor 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 [4]. 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, McCoy's 5A, and F12K culture media Cell culture media with high sodium bicarbonate content can interfere with transduction efficiency [8]. If your cell culture media of choice is affecting transduction efficiency, transduction can be conducted in DPBS for 6 hours and then the cells can be returned to complete cell culture media containing 2 mM sodium butyrate.

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 sensor. For example, a range of 2.5 µL to 40 µL is a good starting range in a 96-well plate (Fig. 6). Choose the range that gives you at least 5-10 times above background and gives you the highest amount of fluorescence change with your Rs control receptor.

Varying the cell density, concentration of sodium butyrate, or trying a new HDAC inhibitor (VPA or TSA) may boost expression as well. optimizing to avoid sensor saturation

Please contact us if you would like to use the sensor under the control of a specific promoter system. Sensors under 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 sensor.

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

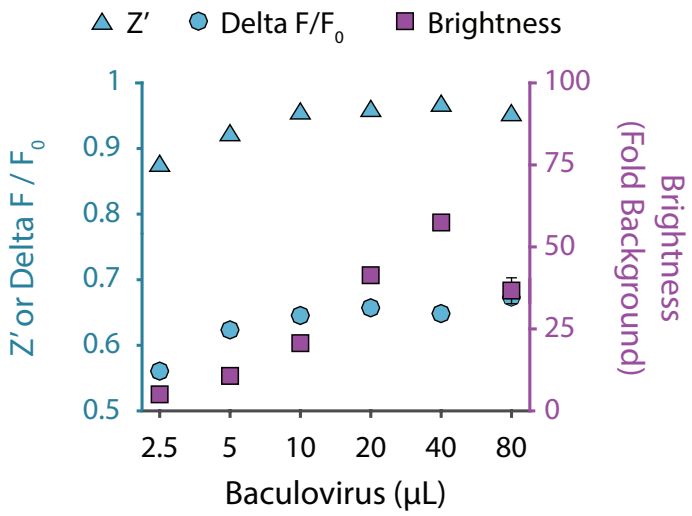


Figure 6. As the amount of cADDis added to the wells increases, so does the baseline fluorescence, plotted in purple. The change in fluorescence when cAMP changes also increases with more virus, but reaches the maximum possible change and remains constant over a broad range of virus concentration (circles). Z' is high over a broad range of virus concentration (triangles).

Use the Positive Control

If the cells are expressing the sensor, and fluorescence is detectable on your instrument, then evaluate sensor performance using the positive control receptor included in your kit. Add 5 µL of the **Rs** (β2 adrenergic control receptor) to a set of control wells and activate with isoproterenol included in the kit (Fig. 5).

Optimizing Receptor Expression

If you have titrated the cADDis sensor and determined the optimal volume, but fail to see a receptor mediated signal, the receptor expression level may be the issue. Try titrating the receptor with a fixed amount of the cADDis sensor.

If you need further help, see the Troubleshooting Guide or let us know, we're happy to help!

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Troubleshooting Guide

Problem	Possible Cause	Solution
1. Low sensor expression and/or poor transduction efficiency	Suboptimal sensor BacMam volume is being used.	Perform titration of the sensor 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 Assays in Adherent Cells.**
	Suboptimal cell density; too few or too many cells added.	Transduce cells so that they are between 80-90% confluent at the time ofthe experiment. 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 45-60 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 DPBS and adding to cells. Replace transduction mix with media after 2-6 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.

	Problem	Possible Cause	Solution
2.	**Low fluorescence signal on microscope/plate reader.**	Low sensor expression, low transduction efficiency.	See solutions for Problem 1.
		Excitation/emission settings are not optimal for the sensor being used.	Refer to protocol for the fluorescence spectra of the sensor. Make sure that filter sets or monochromators are aligned with the peak excitation and emission wavelengths of the sensor.
		Cells are in cell culture media, and the media is producing a large fluorescent signal (autofluorescence).	Exchange media so that cells are in DPBS 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. See our recommended plates.
		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 sensor are not much brighter than control cells/ wells without sensor).**	Low sensor expression, low transduction efficiency.	See solutions for Problem 1.
		Excitation/emission settings are not optimal for the sensor being used.	Refer to protocol for the fluorescence spectra of the sensor. Make sure that filter sets or monochromators are aligned with the peak excitation and emission wavelengths of the sensor.
		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 DPBS. Cell culture media being used has high autofluorescence.	Perform media exchange so that cells are in DPBS 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**.
4.	**Signal is noisy.**	Low sensor expression, low transduction efficiency.	See solutions for Problem 1.
		Gain setting or exposure time on instrument is too low.	Increase gain setting or exposure time.
		Media exchange was not performed, or plate washing was inadequate causing high well-to-well variability. Cells are not in DDPBS at the time of experiment.	Exchange media so that cells are in DDPBS at the time of experiment. If cell culture media must be used, try using FluoroBrite media. Make sure that plate washing is highly consistent from well to well.
		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**.
		Cells are detaching from the plate.	Coat the plate with poly-D lysine or other appropriate cell attachment factor. See our recommended plates

	Problem	Possible Cause	Solution
5.	**Good fluorescence signal, but sensor is not responding to drug as expected. No change in fluorescence observed, or signal is in the wrong direction.**	Photobleaching	Reduce exposure time, sampling rate, and/or light intensity.
		Drug is at the wrong concentration	Confirm drug concentration and solubility.
		Drug was not stored properly.	Confirm drug storage conditions.
		Drug was added to the cells in a volume that was too low relative to the volume of DDPBS/media in the well, resulting in improper mixing.	Add drug in a volume that will allow for sufficient diffusion (i.e. 1:3 or 1:4 drug to total volume)
		Drug was not added in the same solution as the solution in the well/culture dish.	Make sure that the drug preparation and cells are in the same solution.
		Drug addition is producing an artifact.	Make sure to add a vehicle-only control. Make sure drug is added in a solution that is the same as the solution in the well. Do not exceed 1% DMSO final in the well (0.1% or less is ideal).
		Compounds being tested are fluorescent.	Scan compounds for fluorescence to confirm. If possible, dilute compounds in order to reduce the fluorescence artifact of the compound.
		Drug addition was too forceful and dislodged cells.	Add drugs manually or with an on-board dispense function, but do so gently, so as not to dislodge cells.
		Baseline reads were not acquired before adding drug.	Acquire 10-20 baseline fluorescence reads before adding drug. Monitor for a change in fluorescence intensity upon addition of drug.
		Gain setting on instrument is too high, and signal is saturating. Gain setting is too low, and signal cannot be detected.	Adjust gain setting.
		Too much sensor has been added to cells and the signal is saturated (i.e. not enough analyte for the amount of sensor in the cell).	Titrate the amount of sensor to determine maximum signal for your cell type. See protocol recommendations for HEK293T and CHO cells.
		Target receptor was not added, or expression levels are suboptimal (too little or too much, or receptor has high level of constitutive activity).	Titrate the amount of receptor to optimize the signal for your cell type and receptor.
		Sampling rate is not consistent with sensor kinetics.	Acquire 10-20 baseline reads before adding drug. Resume measurement quickly after adding drug (within 5-10 seconds for DAG/PIP_2, 60 seconds for cADDis and cGMP, and 1-2 seconds for GECO Ca^{2+}). Measure long enough to capture max response of sensor.
6.	**Poor cell health, cells detaching from plate.**	Too much BacMam stock was added to cells (e.g. sensor, receptor, Gs mutant).	Titrate lower amounts of BacMam stock to identify the optimal volume for your cells.
		Concentration of HDAC inhibitor is too high, or cells are sensitive to the HDAC inhibitor being used.	Confirm concentration of HDAC inhibitor being used. Make new stock solution. Try a different HDAC inhibitor. Confirm that they are being used 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.
		Plate surface is not coated with a cell attachment factor.	Coat plates with a cell attachment factor (e.g. PDL, laminin, collagen, fibronectin etc.) to enhance attachment.
		Edge wells are being used, and cells in the edge wells may be subject to conditions that are not conducive to growth.	Do not use edge wells.

Problem	Possible Cause	Solution
6. Poor cell health, cells detaching from plate.	Too much BacMam stock was added to cells (e.g. sensor, receptor, Gs mutant).	Titrate lower amounts of BacMam stock to identify the optimal volume for your cells.
	Concentration of HDAC inhibitor is too high, or cells are sensitive to the HDAC inhibitor being used.	Confirm concentration of HDAC inhibitor being used. Make new stock solution. Try a different HDAC inhibitor. Confirm that they are being used 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.
	Plate surface is not coated with a cell attachment factor.	Coat plates with a cell attachment factor (e.g. PDL, laminin, collagen, fibronectin etc.) to enhance attachment.
	Edge wells are being used, and cells in the edge wells may be subject to conditions that are not conducive to growth.	Do not use edge wells.
	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.z
	DDPBS being used does not contain Ca^{2+} and Mg^{2+}.	Use DDPBS containing Ca^{2+} and Mg^{2+}.
	Cells are contaminated.	Monitor cells for bacteria, fungi, mycoplasma.
	Cells were not grown under proper growth conditions (i.e. 5% CO_2, 37°C).	Incubate transduced cells at 37°C, in 5% CO_2.
	Cells are sensitive to acidity from the insect cell culture media present in BacMam virus. The insect cell culture media is more acidic than typical mammalian cell culture media.	Wash transduction mix off of cells after 2-6 hours. Replace with fresh cell culture media, maintaining concentration of sodium butyrate or other HDAC inhibitor.

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. Tewson PH, Martinka S, Shaner N, Hughes TE, Quinn AM: *New DAG and cAMP sensors optimized for live cell assays in automated laboratories.* Journal of Biomolecular Screening 2015.
6. 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.
7. Hoare, S., et al. *Analyzing kinetic signaling data for G-protein-coupled receptors*. Sci Rep. Jul 2020
8. Shen H-C, et al., *Baculovirus-mediated gene transfer is attenuated by sodium bicarbonate.* J Gene Med. Jun. 2007.
9. 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

cADDis - in the Literature

1. N. Philip. Fatty acid metabolism promotes TRPV4 activity in lung microvascular endothelial cells in pulmonary arterial hypertension. Lung Cellular and Molecular Physiology. January 2024.
2. X. Chen, et al. A PACAP-activated network for secretion requires coordination of Ca2+ influx and Ca2+ mobilization. bioRxiv. January 2024.
3. L. Ripoll & M. von Zastrow. Spatial organization of adenylyl cyclase and its impact on dopamine signaling in neurons. bioRxiv. December 2023.
4. T. Richardson, Y. Pettway, et al. Human Pseudoislet System for Synchronous Assessment of Fluorescent Biosensor Dynamics and Hormone Secretory Profiles. Journal of Visualized Experiments. November 2023.
5. C. Hinds, et al. Abolishing β-arrestin recruitment is necessary for the full metabolic benefits of G protein-biased glucagon-like peptide-1 receptor agonists. Diabetes, Obesity and Metabolism. October 2023.
6. E. Blythe & M. von Zastrow. β-Arrestin-independent endosomal cAMP signaling by a polypeptide hormone GPCR. Nature Chemical Biology. September 2023.
7. R. Matt, et al. Fingerprinting heterocellular β-adrenoceptor functional expression in the brain using agonist activity profiles. Frontiers in Molecular Biosciences. August 2023.
8. Y. Kunioku, et al. Intracellular cAMP Signaling Pathway via Gs Protein-Coupled Receptor Activation in Rat Primary Cultured Trigeminal Ganglion Cells. Biomedicines. August 2023.
9. K.M. Semesta, et al. The psychosis risk factor RBM12 encodes a novel repressor of GPCR/cAMP signal transduction. Journal of Biological Chemistry. August 2023.
10. Y.J. Peng, et al. Hypoxia sensing requires H2S-dependent persulfidation of olfactory receptor 78. Science Advances. July 2023.
11. S. Zhang, et al. Competition between stochastic neuropeptide signals calibrates the rate of satiation. bioRxiv. July 2023.
12. E. Kitayama, et al. Functional Expression of IP, 5-HT4, D1, A2A, and VIP Receptors in Human Odontoblast Cell Line. Biomolecules. May 2023.
13. D. Santana Nunez, et al. Piezo1 induces endothelial responses to shear stress via soluble adenylyl Cyclase-IP3R2 circuit. iScience. May 2023.
14. S. Bitsi, et al. Divergent acute versus prolonged pharmacological GLP-1R responses in adult β cell–specific β-arrestin 2 knockout mice. Science Advances. May 2023.
15. V. Bhatia, et al. Characterization of Adenylyl Cyclase Isoform 6 Residues Interacting with Forskolin. Biology. April 2023.
16. H. Carr, et al. The Wnt pathway protein Dvl1 targets Somatostatin receptor 2 for lysosome-dependent degradation. Journal of Biological Chemistry. March 2023.
17. J. Alvarado et al. Transient cAMP production drives rapid and sustained spiking in brainstem parabrachial neurons to suppress feeding. bioRxiv. March 2023.
18. I. Cattani-Cavalieri, et al. Quantitative phosphoproteomic analysis reveals unique cAMP signaling pools emanating from AC2 and AC6 in human airway smooth muscle cells. Frontiers in Physiology. February 2023.
19. E. Porpiglia, et al. Elevated CD47 is a hallmark of dysfunctional aged muscle stem cells that can be targeted to augment regeneration. Cell Stem Cell. December 2022. (bioRxiv)
20. J. Janetzko, et al. Membrane phosphoinositides regulate GPCR-β-arrestin complex assembly and dynamics. Cell. November 2022.
21. N. Saito, et al. Gαs-Coupled CGRP Receptor Signaling Axis from the Trigeminal Ganglion Neuron to Odontoblast Negatively Regulates Dentin Mineralization. biomolecules. November 2022.
22. J. Xu, et al. An evolutionarily conserved olfactory receptor is required for sex differences in blood pressure. bioRxiv. November 2022.

23. B. Barsi-Rhyne, et al. Discrete GPCR-triggered endocytic modes enable β-arrestins to flexibly regulate cell signaling. eLife. October 2022. (bioRxiv)
24. S. A. Dai, et al. State-selective modulation of heterotrimeric Gαs signaling with macrocyclic peptides. Cell. September 2022.
25. J. H. Cho, et al. Islet primary cilia motility controls insulin secretion. Science Advances. September 2022. (bioRxiv)
26. E. Blythe, M. von Zastrow. A discrete mode of endosomal GPCR signaling that does not require β-arrestins. bioRxiv. September 2022.
27. ER McGlone, et al. *Hepatocyte cholesterol content modulates glucagon receptor signaling.* Molecular Metabolism. September 2022.
28. J. Xu, J. Pluznick. *Key amino acids alter activity and trafficking of a well-conserved olfactory receptor.* Cell Physiology. June 2022.
29. C. Zhang, et al. *A brainstem circuit for nausea suppression.* Cell Reports. June 2022.
30. J. Hansen, et al. *A cAMP signalsome in primary cilia drives gene expression and kidney cyst formation.* EMBO Reports. June 2022.
31. S. Ansari, et al. *Morphogen Directed Coordination of GPCR Activity Promotes Primary Cilium Function for Downstream Signaling.* bioRxiv. May 2022.
32. E. Porpiglia, et al. *Elevated CD47 is a hallmark of dysfunctional aged muscle stem cells that can be targeted to augment regeneration.* bioRxiv. April 2022.
33. S. Bitsi, et al. *Divergent acute versus prolonged in vivo GLP-1R responses in β-arrestin 2-deleted primary beta cells.* bioRxiv. April 2022.
34. Y. Mizobuchi, et al. *Ketamine Improves Desensitization of μ-Opioid Receptors Induced by Repeated Treatment with Fentanyl but Not with Morphine.* biomolecules. March 2022.
35. B. Polacco, et al. *Profiling the diversity of agonist-selective effects on the proximal proteome environment of G protein-coupled receptors.* bioRxiv. March 2022.
36. D. Lovinger, et al. *Local modulation by presynaptic receptors controls neuronal communication and behavior.* Nature Reviews Neuroscience. February 2022.
37. F. De Logu, et al. *Schwann cell endosome CGRP signals elicit peri orbital mechanical allodynia in mice.* Nature Communications. February 2022.
38. A. Lutas, et al. *History-dependent dopamine release increases cAMP levels in most basal amygdala glutamatergic neurons to control learning.* Cell Reports. January 2022.
39. G. Sancar, et al. *FGF1 and insulin control lipolysis by convergent pathways.* Cell Metabolism. January 2022.
40. S. Hoare, et al. Quantifying the Kinetics of Signaling and Arrestin Recruitment by Nervous System G-Protein Coupled Receptors. Frontiers in Cellular Neuroscience. January 2022.
41. J. H. Cho, et al. *Islet primary cilia motility controls insulin secretion.* bioRxiv. December 2021.
42. Y. Karasawa, et al. *In Vitro Analyses of Spinach-Derived Opioid Peptides, Rubiscolins: Receptor Selectivity and Intracellular Activities through G-protein- and β-arrestin-Mediated Pathways.* Molecules. October 2021
43. A. White, et al. *Spatial Bias in cAMP generation determines biological responses to PTH type 1 receptor activation.* Science Signaling. October 2021.
44. J. Janetzko, et al. *Membrane phosphoinositides stabilize GPCR-arrestin complexes and offer temporal control of complex assembly and dynamics.* bioRxiv. October 2021.
45. S. Hoare, T. Hughes. *Biosensor Assays for Measuring the Kinetics of G-Protein and Arrestin-Mediated Signaling in Live Cells.* The Assay Guidance Manual. September 2021.
46. Y. Kuroda, et al. *Inhibition of endothelin A receptor by a novel, selective receptor antagonist enhances morphine-induced analgesia: Possible functional interaction of dimerized endothelin A and μ-opioid receptors.* Biomedicine & Pharmacotherapy. September 2021.
47. A. Lutas, et al. *Dopamine-dependent cAMP dynamics in basal amygdala glutamatergic neurons.* bioRxiv. September 2021.
48. F. De Logu, et al. *CGRP Signals from Endosomes of Schwann Cells to Elicit Migraine Pain.* Research Square. August 2021.

49. C. Wu, et al. *Discovery of ciliary G protein-coupled receptors regulating pancreatic islet insulin and glucagon secretion.* Genes & Development. August 2021. bioRxiv.

50. S. Zhang, et al. *Hypothalamic dopamine neurons motivate mating through persistent cAMP signalling.* Nature. August 2021.

51. M. Nuriya, et al. Alkyne-Tagged Dopamines as Versatile Analogue Probes for Dopaminergic System Analysis. Analytical Chemistry. July 2021.

52. A. Gad, et al. Conserved residues in extracellular loop 2 regulate Stachel-mediated activation of ADGRG2. Scientific Reports. July 2021. (bioRxiv)

53. N. Senese, et al. Antidepressants produce persistent Gαs associated signaling changes in lipid rafts following drug withdrawal. Molecular Pharmacology. May 2021.

54. W. Han, et al. Nausea and the Brain: The Chemoreceptor Trigger Zone Enters the Molecular Age. Neuron. February 2021.

55. H. Carr, et al. The PDZ Domain Protein SYNJ2BP Regulates GRK-dependent Sst2A Phosphorylation and Downstream MAPK Signaling. Endocrinology. February 2021.

56. A. Pronin, et al. Ectopically expressed olfactory receptors OR51E1 and OR51E2 suppress proliferation and promote cell death in a prostate cancer cell line. Journal of Biological Chemistry. February 2021.

57. S. Thornquist, et al. *Biochemical evidence accumulates across neurons to drive a network-level eruption.* Molecular Cell. February 2021.

58. Z. Zhou, et al. *Astrocytic cAMP modulates memory via synaptic plasticity.* PNAS. January 2021.

59. M. Draper, et al. *Imaging Meets Cytometry: Analyzing Heterogenous Functional Microscopic Data from Living Cell Populations.* J. Imaging. January 2021.

60. M. Thomas, et al. *Optically activated, customizable, excitable cells.* PLOS One. December 2020.

61. C. Zhang, et al. *Area Postrema Cell Types That Mediate Nausea-Associated Behaviors.* Neuron. November 2020.

62. T. Patriarchi, et al. *An expanded palette of dopamine sensors for multiplex imaging in vivo.* Nature Methods. September 2020.

63. S. Hoare, et al. *Analyzing kinetic signaling data for G-protein-coupled receptors.* Nature Scientific Reports July 2020.

64. J. Hansen, et al. *Nanobody-directed targeting of ontogenetic tools to study signaling in the primary cilium.* eLife. June 2020.

65. J. Yu et al. *N-3 polyunsaturated fatty acids promote astrocyte differentiation and neurotrophin production independent of cAMP in patient-derived neural stem cells.* Mol. Psychiatry. June 2020.

66. J. Cortes-Troncoso, et al. *T cell exosome-derived miR-142-3p impairs glandular cell function in Sjörgen's Syndrome.* JCI Insight. May 2020.

67. I. Gapallawa, et al. *Bitter taste receptors stimulate phagocytosis in human macrophages through calcium, nitric oxide, and cyclic-GMP signaling.* Cell. Mol. Life Sci. March, 2020 (bioRxiv)

68. N. Daram, et al. *Glucagon-like Peptide 1 Shows Glucose Dependence in Regulating Islet Hormone Secretion.* Metabolism. March 2020.

69. C. Koziol-White et al. *Budesonide enhances agonist-induced bronchodilation in human small airways by increasing cAMP production in airway smooth muscle.* American Journal of Physiology. February 2020.

70. S. Hoare, et al. *A kinetic method for measuring agonist efficacy and ligand bias using high resolution biosensors and a kinetic data analysis framework*. Nature Scientific Reports Feb 2020.

71. D. McMahon, et al. *Neuropeptide regulation of secretion and inflammation in human airway gland serous cells.* European Respiratory Journal. Feb 2020 (bioRxiv)

72. R. Forteza, et al. *Glucocorticoids and myosin5b loss of function induce heightened PKA signaling in addition to membrane traffic defects.* Molecular Biology of the Cell. Dec. 2019

73. Nunez, F.J., Johnstone, T.B. et al. *Glucocorticoids rapidly activate cAMP production via Gas to initiate non-genomic signaling that contributes to one-third of their canonical genomic effects.* FASEB Journal, Dec. 2019

74. Nunez, F.J., Schulte, N.A., Fogel, D.M., et al. *Agonist-specific desensitization of PGE2-stimulated cAMP signaling due to upregulated phosphodiesterase expression in human lung fibroblasts.* Naunyn-Schmiedeberg's Arch Pharmacol Dec. 2019

75. K. Harlen, et al. *Live-Cell Assays for Cell Stress Responses Reveal New Patterns of Cell Signaling Caused by Mutations in Rhodopsin, α-Synuclein and TDP-43.* Front. Cell. Neurosci.,December 2019

76. MP Pedro et al. *Activation of G-Protein Coupled Receptor-Gai Signaling Increases Keratinocyte Proliferation and Reduces Differentiation, Leading to Epidermal Hyperplasia*. Journal of Investigative Dermatology. November 2019.

77. K. Hilgendorf, et al. *Omega-3 Fatty Acids Activate Ciliary FFAR4 to Control Adipogenesis.* Cell November, 2019

78. R. Sherpa et al. *Sensory primary cilium is a responsive cAMP microdomain in renal epithelia.* Scientific Reports. April 2019

79. J. Knudsen, et al. *Dysregulation of Glucagon Secretion by Hyperglycemia-Induced Sodium-Dependent Reduction of ATP Production.* Cell Metabolism. February 2019.

80. R. Lee, et al. *Inverse regulation of secretion and inflammation in human airway gland serous cells by neuropeptides upregulated in allergy and asthma.* bioRxiv, May 10, 2019.

81. T. Baldwin, et al. *Insights into the Regulatory Properties of Human Adenylyl Cyclase Type 9.* Molecular Pharmacology, April, 2019.

82. T. Togo *Autocrine purinergic signaling stimulated by cell membrane disruption is involved in both cell membrane repair and adaptive response in MDCK cells.* Biochem & Biophysical Research Comm. , March, 2019.

83. X. Chen, et al. *Phenylephrine, a common cold remedy active ingredient, suppresses uterine contractions through cAMP signalling.* Scientific Reports, Aug. 2018.

84. T. Buranda, et al. *A High-Throughput Flow Cytometry Screen Identifies Molecules that Inhibit Hantavirus Cell Entry.* SLAS Discovery, April 2, 2018.

85. N. H. Wray, et al. *NMDAR-independent, cAMP-dependent antidepressant actions of ketamine.* Molecular Psychiatry, April 2, 2018.

86. H. Zou, et al. *PDE8: A Novel Target in Airway Smooth Muscle.* American Journal of Respiratory Cell and Molecular Biology, Vol. 58, No. 4. April 01 2018.

87. T.B. Johnstone, et al. *PDE8 is Expressed in Human Airway Smooth Muscle and Selectively Regulates cAMP Signaling by β2AR-AC6.* American Journal of Respiratory Cell and Molecular Biology, Dec. 2017.

88. K.A. McCrink, et al. *β-Arrestin2 Improves Post-Myocardial Infarction Heart Failure via Sarco(endo)plasmic Reticulum Ca2+ -ATPase-Dependent Positive Inotropy in Cardiomyocytes*. Hypertension, Nov. 2017.

89. J. Almaça, et al. *Human beta cells produce and release serotonin to inhibit glucagon secretion from alpha cells.* Cell Reports, Volume 17, Issue 12, 2016.

90. P. Tewson, et al. *New DAG and cAMP Sensors Optimized for Live-Cell Assays in Automated Laboratories.* J Biomol Screen, Dec. 11, 2015.

91. P. Tewson, et al. *Assay for Detecting Gai-Mediated Decreases in cAMP in Living Cells.* SLAS, July 10, 2018.

Related Products

Product	Description	Promoter	Recommended Use
#U0200R	Red Up cADDis cAMP	CMV	Fluorescence imaging and plate reader assay (Z' < 0.5)
#X0200G	Gi cADDis cAMP	CMV	Fluorescence imaging and plate reader assay (Z' > 0.7)
#D0300R	Red Down DAG	CMV	Fluorescence imaging and plate reader assay (Z' > 0.7)
#U0300R	Red Up DAG	CMV	Fluorescence imaging and plate reader assay (Z' > 0.5)
#U0600R	Red GECO Ca^{2+}	CMV	Fluorescence imaging and plate reader assay (Z' > 0.5)

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.

US Patent 11,366,114 B2
European Patent Number: EP3065754B1

Our goal is to make your workflow easy and reproducible.

Questions?
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We'd love to hear about your research.

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