



Cilia-targeted cADDIs Sensors

Overview	2
Relevant Products	2
Materials in the Kit	2
Storage	2
Additional Materials Not Supplied	2
BioSafety Considerations	3
Warranty	3
About these Assays	3
Protocol for Use	3
Fluorescence Detection	5
Assay Performance Considerations	5
Contact Us	6
References	6

Overview

The green cADDis sensor (Tewson, 2015) is a fluorescent protein-based sensor for live cell measurement of cAMP in the primary cilium of mammalian cells. The vector carrying this sensor is BacMam, a modified baculovirus, which can be used for delivery to a wide variety of mammalian cell types, including many primary cultures.

In mammalian cells, BacMam expresses only the fluorescent sensor and is a BSL-1 reagent. We recommend that you take the time to do a dilution series of BacMam in your favorite cells, to optimize efficiency in your particular cells and experiments.

Relevant Products

Product	Description	Promoter	Recommended Use
D0201G	Green cilia-targeted cADDis cAMP sensor	CMV	Fluorescence imaging
D0211G	Ratiometric cilia-targeted green cADDis cAMP sensor with red fluorescent tag	CMV	Fluorescence imaging

Materials in the Kit

- Green Cilia-Targeted cADDis cAMP sensor BacMam in TNM-FH Insect Culture Medium (Allele Biotech product #ABP-MED-10001).

Green fluorescent sensor that decreases in fluorescence intensity in response to increases in cAMP.

- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H₂O.

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

- #D0211G is labeled with a red fluorescent tag for ratiometric measurement.

Storage

BacMam stocks should be stored at 4°C and protected from light. Avoid repeated freeze/thaw cycles.

Additional Materials Not Supplied

- Greiner CellCoat (#655946) is our preferred 96-well plate available from VWR.
- Dulbecco's Phosphate Buffered Saline (DPBS) available from VWR [Dulbecco, R. and Vogt, M. 1957].
- Complete culture media specific to your cells. (Please see Assay Performance Section)
- 2-methyl-5hydroxytryptamine (2-M-5HT) a 5HT₃ receptor agonist
- L-858051 a forskolin derivative

BioSafety Considerations

BacMam does not replicate in mammalian cells and expresses only the fluorescent sensor. While it should be handled carefully, in a sterile environment, it is classified as a Biosafety Level 1 (BSL-1) reagent.

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

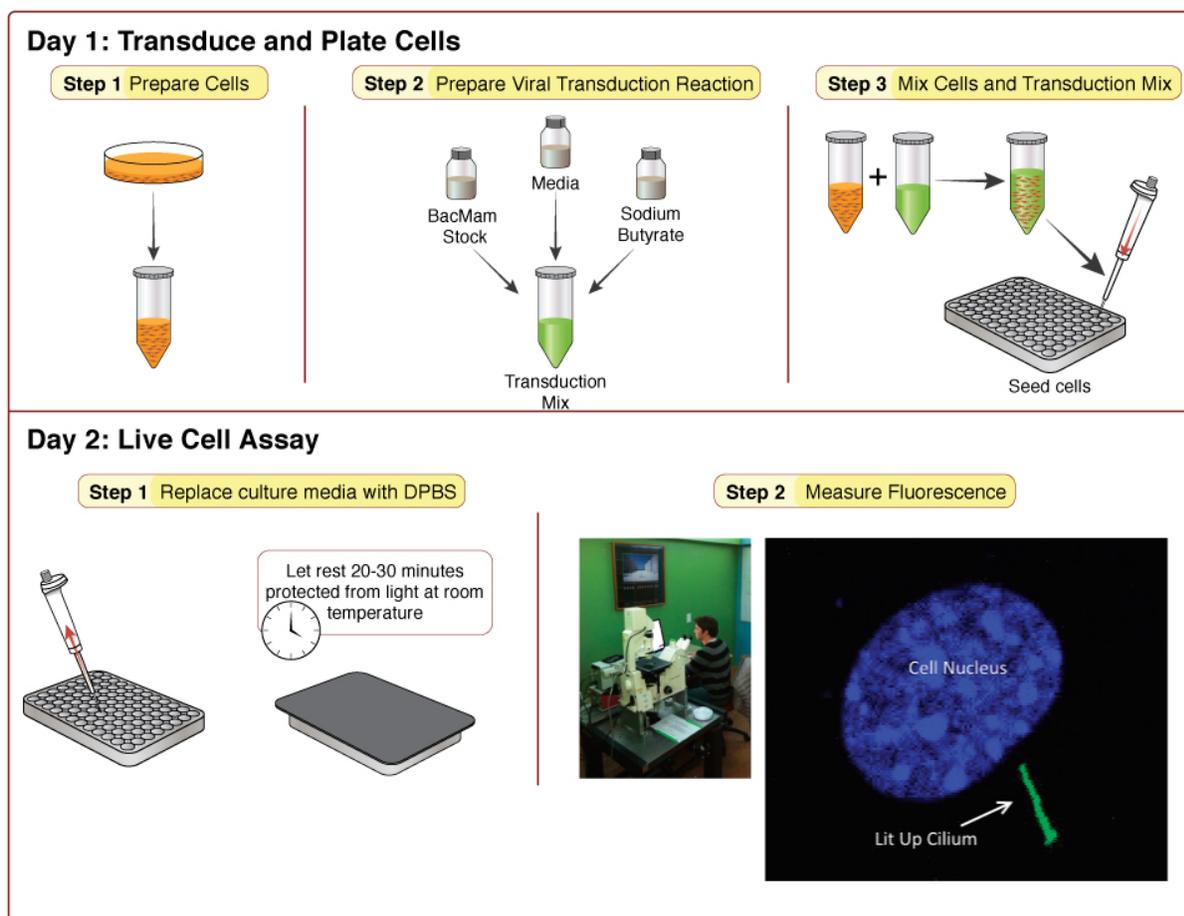
Warranty

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.

About these Assays

Cyclic AMP is an essential second messenger that may regulate pathways inside primary cilia. To measure cAMP within the ciliary space, we fused cADDis, a genetically encoded sensor for cAMP, to the 5HT6 receptor. The cilia-targeted cADDis sensor decreases in fluorescence intensity when localized cAMP is increasing in the cilia. The following protocol assumes cilia formation in your cells (Delling 2013).

Protocol for Use



DAY 1 TRANSDUCE AND PLATE CELLS

Step 1) Prepare cells (Tube A)

- Detach cells from flask using normal trypsinization protocol. Resuspend cells in complete culture media and determine cell count.
- Prepare a dilution of cells at your desired concentration*. 100 μ l 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.

* 450,000 cells/ml works well for HEK293 cells.

Step 2) Prepare Viral Transduction Reaction (Tube B)

- Prepare a 500 mM stock solution of sodium butyrate in sterile water (in your kit).
- For each transduction reaction (i.e. one well in a 96-well plate), prepare the transduction solution by mixing 25 μ l of the BacMam sensor stock with 0.6 μ l of the 500 mM stock solution of sodium butyrate** and 24.4 μ l of the complete culture media for your cells, for a total volume of 50 μ l. Mix gently.

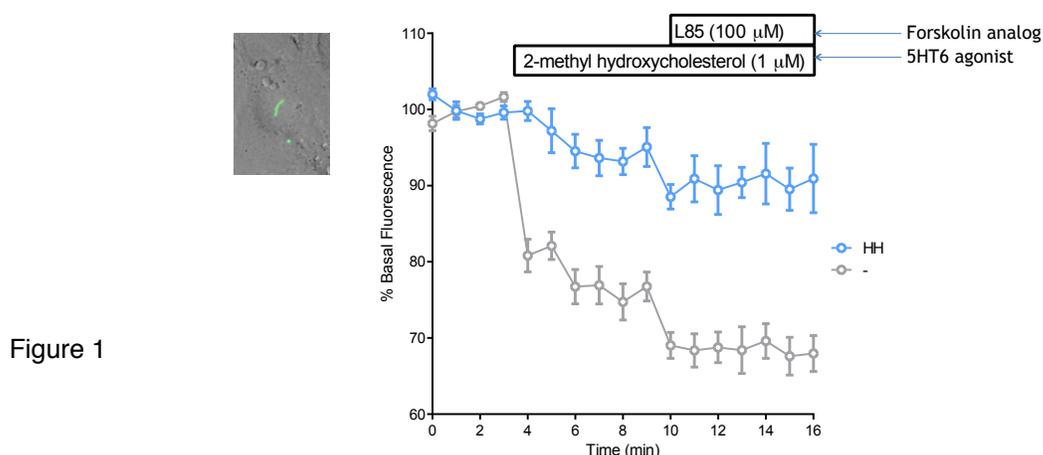
** Concentration of sodium butyrate should be 6mM in this step. Following Step 3, final concentration of sodium butyrate will be 2mM.

Step 3) Mix Cells and Transduction Mix from above.

- Mix Tube A and Tube B (100 μ l tube A + 50 μ l tube B). Mix gently and then seed 150 μ l of mix per well on the 96-well plate.
- Cover plate with aluminum foil to protect from light and incubate at room temperature for 30 minutes.
- Incubate overnight under normal growth conditions, protected from light.

DAY 2 FLUORESCENCE MEASUREMENT

- Cells are now ready for assay. Prior to imaging, replace culture media with DPBS. Wash gently so as not to dislodge cells. **Cover the cells and allow them to rest at room temperature in DPBS for 20-30 minutes before measuring fluorescence.** Experiments are performed at 25°C using standard GFP excitation and emission wavelengths. Figure 1 shows an example of the sensor response in gray in IMCD3 cells, imaged on the left (Moore 2016).



Fluorescence Detection

Fluorescence Properties

This sensor is constructed using a green fluorescent protein [3]. The optimal excitation wavelength is 480 nm, but the absorption band of this protein is quite broad, so broad bandpass filters that pass 450 to 480 nm light can be used quite effectively. On the emission side, the green light spans 510 to 550 nm, so broad band pass emission filters can also help to collect much of the emission. These filter properties are quite similar to many of the FITC filter sets commonly available on most microscopes and plate readers.

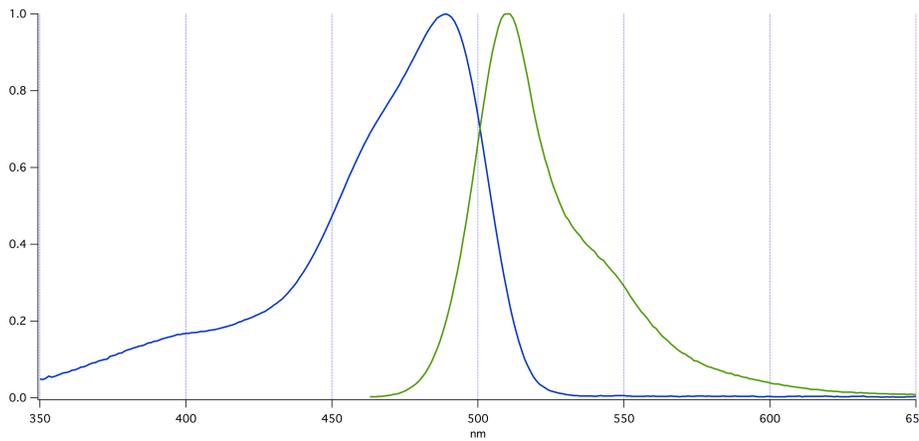


Figure 3. The absorption and emission properties of a typical green fluorescent protein are plotted above as a function of wavelength. Optimal excitation light ranges from 450 to 490 nm, while optimal emission filters should select the emitted light between 510 and 550 nm.

Assay Performance Considerations

Expression levels of the sensor.

To optimize the assay in your particular cell type, it is important to optimize 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.

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 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.

Culture Media Considerations

This assay has been validated with EMEM, DMEM, and F-12K complete growth media. Other types of media may affect results.

Contact Us

If you have ideas about how we can improve our products or protocols, then we would like to hear from you. Your feedback is extremely valuable. Please send an email to info@montanamolecular.com. We'll respond as quickly as we can.

References

1. 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.
2. Delling M, DeCaen PG, Doerner JF, Febvay S., Clapham DE, Primary cilia are specialized calcium signalling organelles. *Nature*. 504, 311-314 (2013).
3. Borner S, Schwede F, Schlipp A, Berisha F, Calebiro D, et al. (2011) FRET measurements of intracellular cAMP concentrations and cAMP analog permeability in intact cells. *Nat Protoc* 6(4): 427-438. 10.1038/nprot.2010.198 [doi].
4. Moore, B.S. et.al. Cilia Have High cAMP Levels That Are Inhibited by Sonic Hedgehog Regulated Calcium Dynamics. *PNAS* 2016, vol. 113 no. 46. doi: 10.1073/pnas.1602393113

PCT/US14/63916 Patent Pending