Montana Molecular
Fluorescent Biosensors for Live Cell Discovery

ArcLight D0700G and D0701G

Overview

This protocol is optimized for measuring cells plated in 96-well format and is appropriate for live-cell imaging. Specific details of the protocol will vary by cell type, so it is important to take the time to titrate BacMam for optimal results in your particular cells. For expression in rare cell types, or specific cells in mixed cultures, ask us about Cre-dependent or specific promoter systems.

The vector carrying these sensors is a modified baculovirus. In mammalian cells, BacMam expresses only the fluorescent sensor and is a BSL-1 reagent. If you want to measure voltage in cells other than excitable HEK 293 cells, then this protocol can be used as a starting point for assay optimization. We recommend that you take the time to do a dilution series in your cells, to optimize in your particular cells and fluorescence detection system.

Relevant Products

Product	Description	Promoter	Recommended Use
D0700G	Green ArcLight Sensor	CMV	Fluorescence imaging
D0701G	Green ArcLight Sensor	Synapsin	Fluorescence imaging

Materials included

- ArcLight voltage sensor BacMam in TNM-FH Insect Culture Medium (Allele Biotech product #ABP-MED-10001). A green fluorescent sensor that decreases in response to voltage changes.

- Sodium Butyrate (Sigma Aldrich product number B5887) 500 mM in H_2O. Sodium Butyrate maintains BacMam expression in cells. Other HDAC inhibitors such as Trichostatin A or valproic acid may be substituted.

Storage

BacMam stock should be stored at 4°C and protected from light. Avoid freezing and thawing.

Additional materials not supplied

- Greiner CellCoat (#655946) is our preferred plate for this assay.
- Dulbecco's Phosphate Buffered Saline (DPBS) available from VWR [Dulbecco, R. and Vogt, M.1957].

BioSafety Considerations

BacMam does not replicate in mammalian cells. While it should be handled carefully, in a sterile environment, it is classified as Biosafety Level 1 (BSL-1). This product is for research use only and is not intended for use 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 the Assay

ArcLight is a genetically-encoded, fluorescent voltage sensor in a BacMam viral delivery vector. See More about the ArcLight voltage sensor here: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0081295

Protocol for Use

Seed cells in appropriate complete growth medium. Allow cells to grow under normal growth conditions until desired conditions are met.

DAY 1

1. Prepare a 500 mM stock solution of sodium butyrate in sterile water.
2. Prepare Transduction Solution*

EXAMPLE: HEK 293 cells in a 96 Well Plate **

Single Well	*Master Mix ******
25 µl Bacmam Stock	x **110** = 2750 µL
23.5 µl DPBS	x **110** = 2,585 µL
1.5 µl 500 mM Na-Butyrate	x **110** = 165 µL
50 µl total volume	

3. Add 50 µl transduction solution to 100 µl media + cells in the well. Mix the solution gently.
4. 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, protected from light, for 6 hours (5% CO_2 and 37°C).
5. 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 24 hrs before measuring fluorescence as described below.

DAY 1 NOTES

* Final concentration of sodium butyrate is 5mM in Step 2. Following Step 3, final concentration of sodium butyrate will be 2mM.

** To optimize results in your cell type, prepare a dilution series by varying the amount of BacMam. For example, a range of 10 uL to 80 uL, adjusting the amount of DPBS accordingly to optimize sensor expression and cell health for your cells. Ask us about purified stock to boost transduction efficiency in primary cultures if needed.

***Master Mix scaled up by ~15% to account for pipetting loss.

DAY 2

Prior to imaging, replace culture media with DPBS. Experiments are performed at 25°C using standard GFP excitation and emission wavelengths. Image fast voltage changes using sensitive, fast cameras (such EMCCD or CMOS cameras), or similarly sensitive devices.

References

Jin, L, et.al. (2012). Single Action Potentials and Subthreshold Electrical Events Imaged in Neurons with a Fluorescent Protein Voltage Probe. Neuron 75, 779-785.

Han, Z. (2013) Fluorescent Protein Voltage Probes Derived from ArcLight that Respond to Membrane Voltage Changes with Fast Kinetics. PLOS One 8(11): e81295.