Montana Molecular
Fluorescent Biosensors for Live Cell Discovery

Arclight BacMam Assay

Overview

This protocol is optimized for measuring cells plated in 96-well format and is appropriate for live-cell imaging. Greiner CellCoat (#655946) is our preferred plate. 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 availability of a Cre-dependent or specific promoter system.

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 DAG in cells other than HEK 293, then the IU/mL needed to transduce a well of HEK 293 cells can be used as a starting point for assay optimization in other cell types. 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).
  Green fluorescent sensor that decreases in response to voltage changes.

- Sodium Butyrate (Sigma Aldrich product number B5887) 500 mM in H2O.
  Sodium Butyrate maintains BacMam expression in cells. Other HDAC inhibitors such as Trichostatin A (TSA) or Valproic acid (VPA) may be substituted.

Storage

Baculovirus stock should be stored at 4°C and protected from light. Avoid freeze/thaw cycles.

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 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

A genetically-encoded, fluorescent voltage sensor in a BacMam viral delivery system. A noninvasive, easy-to-use tool for studying the electrical activity of live neurons. To learn more about the development of voltage sensors, take some time to visit Vincent Pieribone's wonderful pages dedicated to fluorogenic voltage sensors: http://fluorogenetic-voltage-sensors.org.

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. For each transduction reaction (i.e. one well in a 96-well plate,100uL per well), prepare a transduction solution by mixing 25 uL of the BacMam 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.
3. Prepare a dilution series of transduction reactions 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.

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 above

DAY 2

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 using standard GFP excitation and emission wavelengths.

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

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