Montana Molecular
Fluorescent Biosensors for Live Cell Discovery

Kit Insert

Relevant Product Numbers

#U0200G	#U0200R	#D0231G	#D0250G	#U0261G	#U0266G
#U0205G	#U0205R	#D0241G	#U0255G	#U0262G	#U0267G
#D0200G	#D0221G	#U0241R	#D0255G	#U0263G	
#D0205G	#U0221R	#U0250G	#U0250R	#U0264G	

Materials included

- cADDis sensor for cAMP. BacMam vector: ~2x10^{10} VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

  Fluorescent sensor that changes in fluorescence intensity in response to changes in cAMP.

- β2 Adrenergic receptor (Gs-coupled GPCR). BacMam vector: ~10^{10} VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems product #96-001-01).

- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H_2O.

  Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. Some cell types maintain expression without an HDAC inhibitor.

- Isoproterenol agonist control (Sigma Aldrich Product Number I2760) 10 mM in 10 mM HCl

  Agonist activates β2 Adrenergic receptor signaling and results in robust fluorescence change.

Storage

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. We recommend retesting BacMam stock after storing for more than 12 months at 4°C, or after any freeze-thaw cycle. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

QA/QC

BacMam stocks are tested for sterility before packaging. Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO_2, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

Biosafety

BacMam is the modified baculovirus, *Autographa californica*, AcMNPV. Baculovirus is pseudotyped to infect mammalian cells, but it cannot replicate in the cells and its genome is silent in mammalian cells. While it should be handled carefully, in a sterile environment, it is classified as a BSL-1 reagent. The NIH Guidelines for Research on Recombinant DNA Molecules should be consulted for laboratory safety procedures.

ESF 921™ Insect Cell Culture Medium is a complete serum-free, protein-free medium. ESF 921™ contains L-Glutamine and Kolliphor® P188 (Pluronic F68).

For Research Use Only. Not recommended for use or sale in human or animal diagnostic or therapeutic products. This product contains no substances which at their given concentration are considered to be hazardous to health, however we recommend handling with care. Wear impervious gloves and eye protection when handling. Do not ingest.

Review the protocols on Montana Molecular's Website: www.montanamolecular.com before using these products.

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 any components of these products is conveyed. Reverse engineering or modification of these products is not permitted.

Made in the USA

European Patent Number: EP3065754B1

Patent: PCT/US2014/063916 [PCT Issued, US pending National Phase]

Montana Molecular
Fluorescent Biosensors for Live Cell Discovery

Relevant Products

#X0200G #X0205G #X0250G #X0255G

Materials included

- Green Down cADDis sensor for cAMP. BacMam vector: ~2x10^{10} VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

  Fluorescent sensor increases in fluorescence intensity in response to inhibition of cAMP production.

- hD2 Receptor (Gi-coupled GPCR). BacMam vector: ~10^{10} VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems product #96-001-01).

  Use this receptor in control experiments to activate Gi signaling. Other Gi-coupled receptors of interest may either be present in your cell line, or delivered via transduction/viral vector, or via plasmid/transfection.

- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H_2O.

  Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. Some cell types maintain expression without an HDAC inhibitor.

- Quinpirole hydrochloride in 2 mM in Sterile Water

  Activates Gi signaling through the hD2 positive control receptor.

Storage

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

QA/QC

BacMam stocks are tested for sterility. Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO_2, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

Biosafety

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Relevant Products

#D0201G Green Cilia-Targeted cADDis cAMP

#D0211G Ratiometric Cilia-Targeted Green cADDis cAMP

Materials included

- cADDis sensor for cAMP targeted to primary cilia. BacMam vector. ~ $2x10^{10}$ VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

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

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

- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H_2O.

  Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. Some cell types maintain expression without an HDAC inhibitor.

Storage

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO_2, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

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Fluorescent Biosensors for Live Cell Discovery

Relevant Products

#D0251G Green Downward cADDis cAMP Assay (Synapsin)
#U0251G Green Upward cADDis cAMP Assay (Synapsin)

Materials included

- cADDis sensor for cAMP BacMam Vector: ~2x10^{10} VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

  Fluorescent sensor that changes in fluorescence intensity in response to changes in cAMP.

- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H_2O.

  Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. Some cell types maintain expression without an HDAC inhibitor.

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