

SAFETY DATA SHEET

Relevant Products

#D0300R Red Down DAG (CMV)	#U0300R Red Up DAG (CMV)
#D0300G Green Down DAG (CMV)	#D0305G Green Down DAG (CAG)
#U0300G Green Down DAG (CMV)	#U0305G Green Down DAG (CAG)

Materials Included

- Genetically-encoded fluorescent sensor. BacMam vector: $\sim 2 \times 10^{10}$ VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- hM1 muscarinic (Gq-coupled GPCR receptor). BacMam vector: 2×10^{10} in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01). Use this receptor in control experiments.
- Sodium Butyrate (Sigma Aldrich product number 303410) 500 mM in H₂O..

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.

- Carbachol 25 mM in H₂O (Sigma Aldrich product number C4382)

Carbachol stimulates G_q signaling through the hM1 receptor control.

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 agonist controls at -20 °C. Store HDAC inhibitor at 4°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% CO₂, 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. 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, polyhedron minus strain. 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

U.S. Patent No. 9,547,017B2 and 10,131,960
European Patent Pending; EP2825887A4

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#D0400G Green PIP ₂ assay (CMV)	#D0405G Green PIP ₂ assay (CAG)
#D0400R Red PIP ₂ assay (CMV)	#U0608G Green GECO Ca ²⁺ (Ef1 α)
#U0600R Red GECO Ca ²⁺ (CMV)	#U0600G Green GECO Ca ²⁺ (CMV)

Materials Included

- Genetically-encoded fluorescent sensor. BacMam vector: ~ 2x10¹⁰ VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
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