Product Information Sheet

BacMam Aequorin Kit

Catalog Numbers: A13740 and A13741
Literature Lot Number: V1

Literature Part Number: A13741.PIS
Revision date: 2 June 2011

FAST FACTS

BacMam Aequorin utilizes BacMam technology to transiently deliver aequorin, a luminescent calcium sensor, into mammalian cells.

Aequorin has been used extensively to monitor calcium changes in cultured, living mammalian cells and has become a standard method for interrogating calcium second messenger pathways in a high-throughput screening formats.

Upon binding of intracellular calcium ions, aequorin displays a "flash" style luminescence signal while consuming coelenterazine h as a substrate. The luminescence signal resolves any problems with autofluorescent compounds as well as providing an exceptional signal-to-noise ratio.

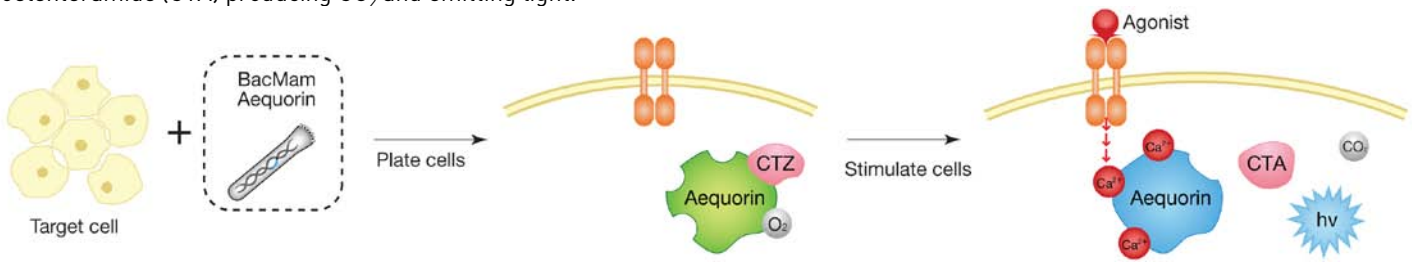
Due to the variation of cell types in which this product may be used, assay optimization may be required for best results (see BacMam Aequorin Transduction Guidelines, next page)

Refer to Kost, T.A., *et al. Drug Disc Today* 2007, *12,* 396–403 for examples of BacMam gene expression in cells. For additional information on BacMam visit www.invitrogen.com/bacmam.

Kit components	Amount A13740	Amount A13741	Storage	Handling
BacMam Aequorin Reagent	10 mL	100 mL	4°C	• Do not freeze • Minimize exposure to ambient light • Use aseptic technique • Aliquot to minimize handling, if necessary
Enhancer Solution	400 µL	4 mL	–20°C	• Protect from light

Additional materials required, but not provided	Source	Part number
Coelenterazine h	Invitrogen	C6780
Opti-MEM® I Reduced Serum Medium	Invitrogen	11058-021
Cell line of interest	Various	Various

Figure1 Aequorin Mechanism of Action. Aequorin-transduced cell lines are loaded with coelenterazine h (CTZ). Upon ligand-binding, intracellular calcium concentrations rise. The free calcium binds to the aequorin molecule leading to oxidization of CTZ to coelenteramide (CTA) producing CO_2 and emitting light.



BacMam Aequorin Transduction Guidelines

Due to the variation of cell types in which this product may be used, assay optimization may be required for best results.

- We recommend performing a virus titration to determine the optimal percentage of virus for transducing your cell line. Test a range of v/v dilutions of the BacMam reagent such as 50%, 25%, 12.5%, 6.25%, and 3.13% (v/v) for initial optimization.
- For difficult-to-transduce cells, such as primary and stem cell lines, we recommend following the transduction protocol described for BacMam GFP control (Catalog no. B10383)
- During the optimization process, we recommend incubating the cells after BacMam transduction in the presence and absence of the 1X BacMam Enhancer Solution. For cell lines such as CHO, HEK293T and HeLa, using the BacMam Enhancer Solution greatly increases the target expression. For certain cell types such as U2-OS, the Enhancer Solution may be toxic or non-beneficial. Add the enhancer solution to the cells during the 16–24 hours incubation period.
- For the best possible assay performance, we recommend optimizing the number of cells per well. For most cell lines, 2,000–10,000 cells per well in 25 µL of medium in 384-well format is optimal.
- We recommend a clear-bottom plate when using instruments with bottom-read capabilities and a solid-bottom plate when using instruments with top-read capabilities.
- To optimize coelenterazine h loading time and temperature, we recommend testing 2, 4, 6, 8, and 24-hour loading times at room temperature and 37°C. Some cell lines can tolerate an overnight incubation at 37°C before significant signal intensity is lost.

Transduction Procedure

1. Add the following to a T-75 or 10-cm culture dish (both have approximately 75 cm^2 of surface area): 4 million cells, 9 mL of culture medium (with or without serum) and 1 ml (10%) BacMam Aequorin reagent.
2. Incubate the cells at 37ºC and 5% CO_2 for 16–24 hours to allow for expression of aequorin protein.
3. Dissociate the cells from flask or dish by trypsinization or other method.
4. Count the cells using a hemacytometer or the Countess® Automated Cell Counter.
5. Centrifuge the cells and resuspend them at 1 million cells per 2.5 mL of Opti-MEM® I Reduced Serum Medium supplemented with 1% charcoal-dextran stripped FBS.

   Note: (Optional) At this stage, cells can be cryopreserved and stored in liquid nitrogen for future use.
6. Add coelenterazine h to a final concentration of 5 µM (1X). Mix well.
7. Plate the cells in the desired format (i.e., 96-, 384-, or 1,536-well plates) at one-half the working volume of the plate type (i.e., 25 µL in a standard 384-well plate with a working volume of 50 µL) using 10,000 cells/well at 25-µL volume in a 384-well format.
8. Incubate the cells at 37°C or room temperature for 2–4 hours.

Calcium Mobilization Assay

Due to the "flash" style luminescence of aequorin, a luminometer with liquid handling injectors is needed. Examples include Hamamatsu FDSS6000 with luminescence option, Hamamatsu FDSS7000, MDS Analytical Technologies FLIPR Tetra® with luminescence option, Perkin Elmer's LumiLux® and LumiLux® CS, CyBio CyBi®-Lumax or the BMG Labtech FLUOstar OPTIMA.

1. Make dilutions of test compounds in Opti-MEM® I Reduced Serum Medium at a 2X concentration.

   Note: A calcium mobilization experiment is typically performed by first collecting a baseline reading for approximately 10 seconds before initiating calcium mobilization by compound addition.
2. Add an equal volume of compound to the cells in the plate (i.e., 25 µL of compound to 25 µL of cells in a 384-well plate).

   Note: The aequorin signal should appear immediately after compound addition and only last for a few seconds
3. Monitor the luminescent signal for 45 seconds after adding the compound.
4. Process the data by computing the area under the curve and plotting that value versus compound concentration.

Technical Support

For additional assistance in using BacMam Aequorin Reagent, contact Technical Support. Email: probestech@invitrogen.com or phone (800) 438-2209 or (541) 335-0353.

Frequently Asked Questions

Visit www.invitrogen.com and search for A13740 or A13741 to download answers to Frequently Asked Questions (FAQs) for this product. The FAQ sheet is located under the "How to Use" tab on the product page.

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