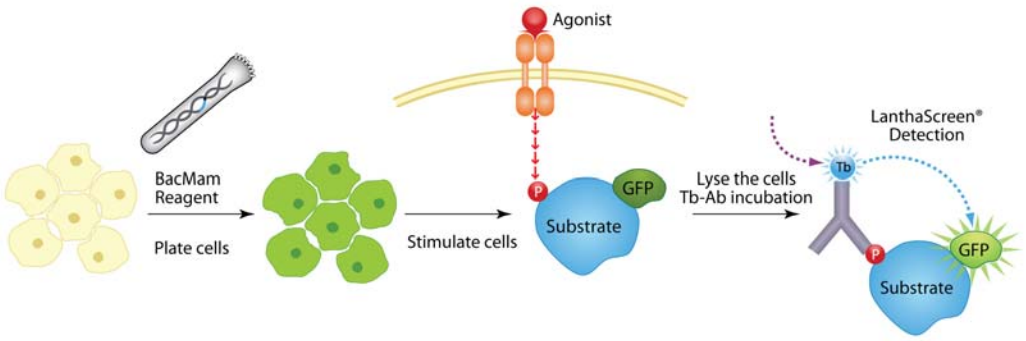
BacMam Histone H3 [AcLys9] Cellular Assay

Overview

Introduction to BacMam. LanthaScreen® Cellular Assays can now be used together with a gene delivery method known as BacMam for the transient expression of the desired GFP-substrate fusion in many cell lines of interest. The BacMam delivery system utilizes modified baculovirus as a vehicle to deliver and express target genes in mammalian cells. This technology has several advantages over commonly used transient methods for heterologous gene expression, including: 1) high transduction efficiency across a broad range of cell types, including primary and stem cells; 2) little-to-no observable cytopathic effects; 3) reproducible and titratable target gene expression; 4) virus is non-replicating in mammalian cells, therefore it is designated as Biosafety Level 1 (BSL1); and 5) compatible with simultaneous delivery of multiple genes. Thus, combination of the LanthaScreen® Cellular Assay technology and BacMam-mediated gene delivery provides a fast, convenient, and robust method for interrogating specific signal transduction events in a cell background of choice. Please see Kost, et al for more information pertaining to BacMam gene expression in cells.



Schematic Illustration of the workflow of BacMam-enabled Cellular Assay Using LanthaScreen® Technology. Cells are treated with BacMam virus encoding a GFP-fusion protein and plated in a 384-well assay plate. 24-48 hours post-transduction, the cells are stimulated to induce the specific post-translational modifications (such as phosphorylation as shown) of the GFP-substrate fusion. Cells are then lysed in the presence of terbium-labeled anti-modification specific antibody and TR-FRET from terbium to GFP can be detected.

***BacMam-enabled Cellular Histone H3 Assay Using LanthaScreen® Technology*.** The eukaryotic nucleosome, composed of histones H2A, H2B, H3, and H4, regulates the structure of chromatin and consequently modulates gene transcription profiles in a concerted manner. Nucleosome function is directly regulated by a multitude of posttranslational modifications on amino-terminal tails of core histones, including acetylation, phosphorylation, methylation and ubiquitination. The combination of baculovirus-mediated gene delivery (BacMam) with LanthaScreen® Cellular Assay technology enables a platform for the analysis of specific posttranslational modifications of histones. BacMam provides a convenient genetic delivery tool for a GFP-Histone H3 fusion protein in the cell line of interest. BacMam-enabled Histone H3 assay is an HTS-compatible cellular immunoassay measuring acetylation of GFP-Histone H3 at Lys9.

Validation Table of Contents

Validation experiments of BacMam Histone H3 [AcLys9] Cellular Assay were performed in a 384-well format using U-2 OS as a host cell line. Following treatment with Trichostatin A [TSA], the acetylation status of GFP-Histone H3 was then measured using LanthaScreen® Tb-anti-Histone H3 [acLys9] antibody as a detection reagent. Included here are representative results using this assay, including an example of the assay optimization process, which can be applied to your cell line of choice.

1. Representative assay optimization and validation for Histone H3 AcLys9 assay using U-2 OS cells

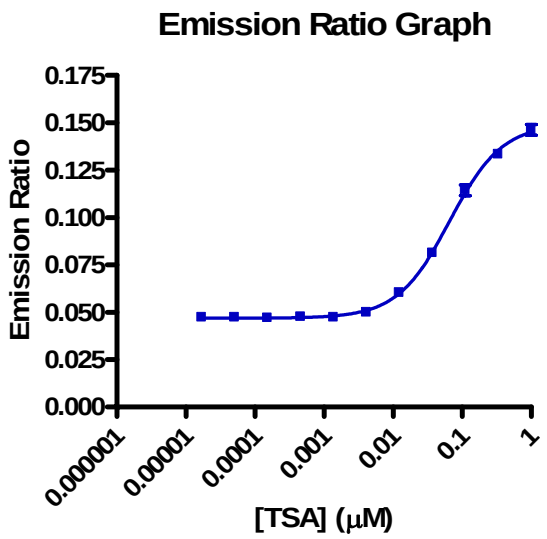
- a. Comparison of emission ratio and response ratio graphs
- b. Optimization of BacMam Histone H3 transduction
- c. Optimization of cell number per well
- d. Optimization of stimulation time
- e. Lysis buffer equilibration time
- f. Alternate compound profiles
- g. Cell cycle dependent acetylation
- h. Localization of GFP-Histone H3

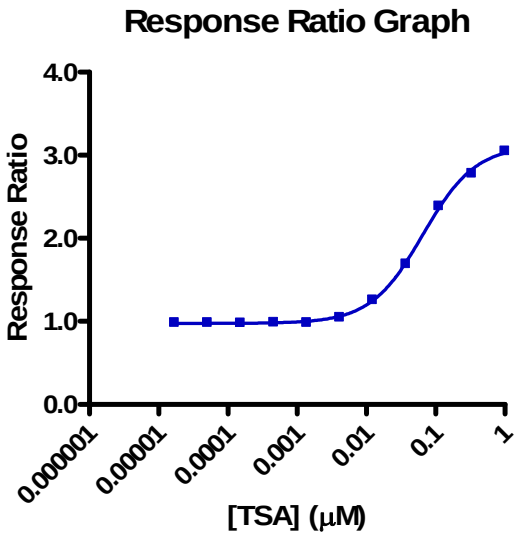
2. Assay Portability

- a. Alternate cell backgrounds tested: 786-O, HCT116, HeLa, NIH-3T3, HDFn

Representative Data for BacMam Histone H3 [AcLys9] Cellular Assay

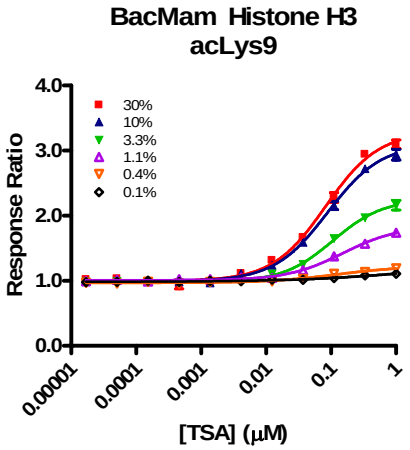
Figure 1a — Comparison of emission ratio and response ratio graphs





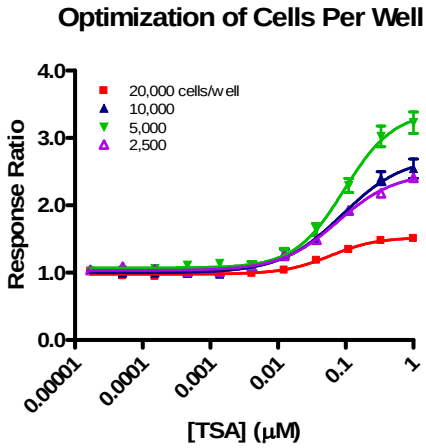
Measurement of Histone H3 acetylation using the BacMam Histone H3 [acLys9] Cellular Assay. U-2 OS cells were harvested, resuspended in assay medium (Opti-MEM I + 0.1% charcoal-stripped FBS) and mixed with BacMam Histone H3 Reagent to achieve 10% virus (v/v) concentration. The mix was applied to wells of a 384-well plate, yielding a cell density of ~5,000 cells per well. Following a 24 hour incubation, cells were treated for 3 hours with a serial dilution of Trichostatin A. Cells were lysed by adding fully-supplemented 6X lysis buffer containing Tb-anti-Histone H3 [acLys9] antibody and equilibrated for 3 hours at room temperature before TR-FRET signal was measured on a BMG Labtech Pherastar plate reader. Raw TR-FRET Emission Ratios (520nm/490nm) and Response Ratios (measure of assay window) normalized to untreated controls are shown.

Figure 1b — Optimization of BacMam Histone H3 transduction



BacMam Histone H3 virus titration. U-2 OS cells were harvested, resuspended in assay medium and mixed with BacMam Histone H3 Reagent to achieve the indicated % virus (v/v) concentrations. The mixes were applied to designated wells of a 384-well plate, yielding a cell density of ~5,000 cells per well. Following a 24 hour incubation, cells were treated for 3 hours with a serial dilution of Trichostatin A. Cells were lysed by adding fully-supplemented 6X lysis buffer containing Tb-anti-Histone H3 [acLys9] Antibody and equilibrated for 3 hours at room temperature before TR-FRET was measured on a BMG Labtech Pherastar plate reader.

Figure 1c – Optimization of cell number



Measurement of Histone H3 acetylation using the BacMam Histone H3 [acLys9] Cellular Assay. U-2 OS cells were harvested, resuspended in assay medium, and serial dilutions of the cells were mixed with BacMam Histone H3 Reagent to achieve 10% virus (v/v) concentration. The mixes were applied to designated wells of a 384-well plate, yielding varying cell densities per well. Following a 24 hour incubation, cells were treated for 3 hours with a serial dilution of Trichostatin A. Cells were lysed by adding fully-supplemented 6X lysis buffer containing Tb-anti-Histone H3 [acLys9] Antibody and equilibrated for 3 hours at room temperature before TR-FRET was measured on a BMG Labtech Pherastar plate reader.

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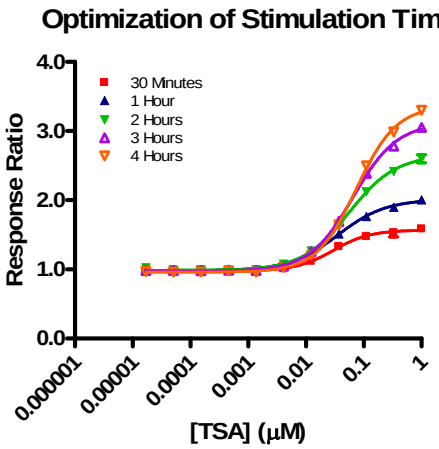
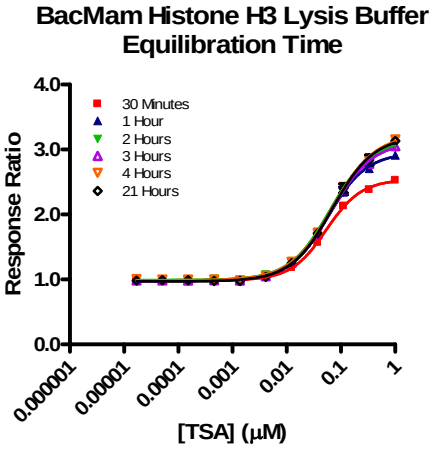
Figure 1d — Optimization of cell stimulation time



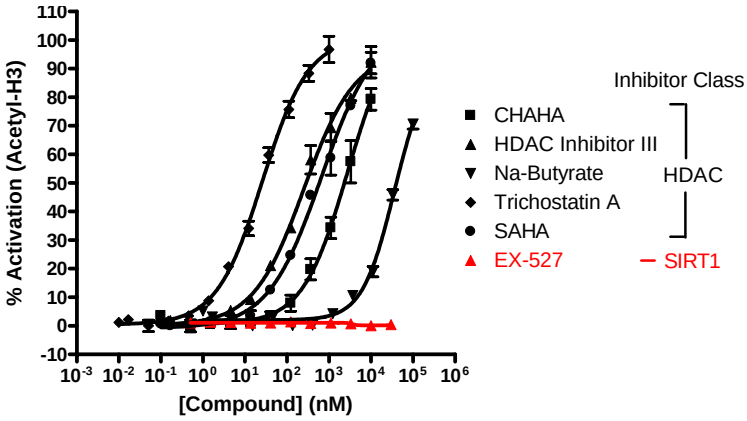
Measurement of Histone H3 acetylation using the BacMam Histone H3 [acLys9] Cellular Assay. U-2 OS cells were harvested, resuspended in assay medium and mixed with BacMam Histone H3 Reagent to achieve 10% virus (v/v) concentration. The mix was applied to wells of a 384-well plate, yielding a cell density of ~5,000 cells per well. Following a 24 hour incubation, cells were treated for the indicated times with a serial dilution of Trichostatin A. Cells were lysed by adding fully-supplemented 6X lysis buffer containing Tb-anti-Histone H3 [acLys9] Antibody and equilibrated for 3 hours at room temperature before TR-FRET was measured on a BMG Labtech Pherastar plate reader.

Figure 1e — Optimization of lysis buffer equilibration time



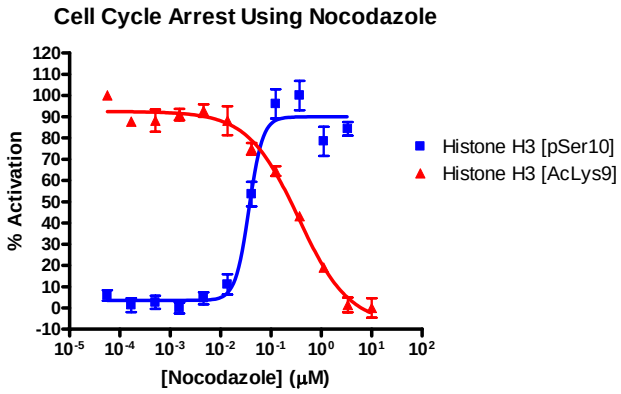
Measurement of Histone H3 acetylation using the BacMam Histone H3 [acLys9] Cellular Assay. U-2 OS cells were harvested, resuspended in assay medium and mixed with BacMam Histone H3 Reagent to achieve 10% virus (v/v) concentration. The mix was applied to wells of a 384-well plate, yielding a cell density of ~5,000 cells per well. Following a 24 hour incubation, cells were treated for 3 hours with a serial dilution of Trichostatin A. Cells were lysed by adding fully-supplemented 6X lysis buffer containing Tb-anti-Histone H3 [acLys9] Antibody and equilibrated for the indicated times before TR-FRET was measured on a BMG Labtech Pherastar plate reader.

Figure 1f — Alternate compound profiles



Measurement of Histone H3 acetylation using the BacMam Histone H3 [acLys9] Cellular Assay. U-2 OS cells were harvested, resuspended in assay medium and mixed with BacMam Histone H3 Reagent to achieve 10% virus (v/v) concentration. The mix was applied to wells of a 384-well plate, yielding a cell density of ~5,000 cells per well. Following a 24 hour incubation, cells were treated for 3 hours with serial dilutions of indicated HDAC inhibitors. Cells were lysed by adding fully-supplemented 6X lysis buffer containing Tb-anti-Histone H3 [acLys9] Antibody and equilibrated for 3 hours at room temperature before TR-FRET was measured on a BMG Labtech Pherastar plate reader.

Figure 1g — Cell cycle-dependent acetylation



Measurement of Histone H3 acetylation using the BacMam Histone H3 [acLys9] Cellular Assay. U-2 OS cells were harvested, resuspended in assay medium and mixed with BacMam Histone H3 Reagent to achieve 10% virus (v/v) concentration. The mix was applied to wells of a 384-well plate, yielding a cell density of ~5,000 cells per well. Following a 24 hour incubation, cells were treated for 3 hours with serial dilutions of nocodazole. Cells were lysed by adding fully-supplemented 6X lysis buffer containing Tb-anti-Histone H3 [acLys9] antibody or [pSer10] antibody and equilibrated for 3 hours at room temperature before TR-FRET was measured on a BMG Labtech Pherastar plate reader.

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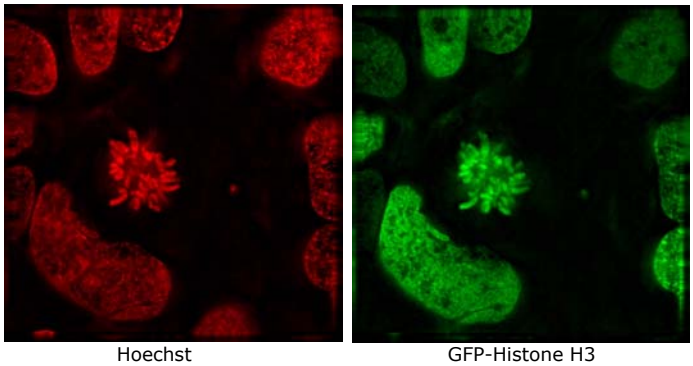
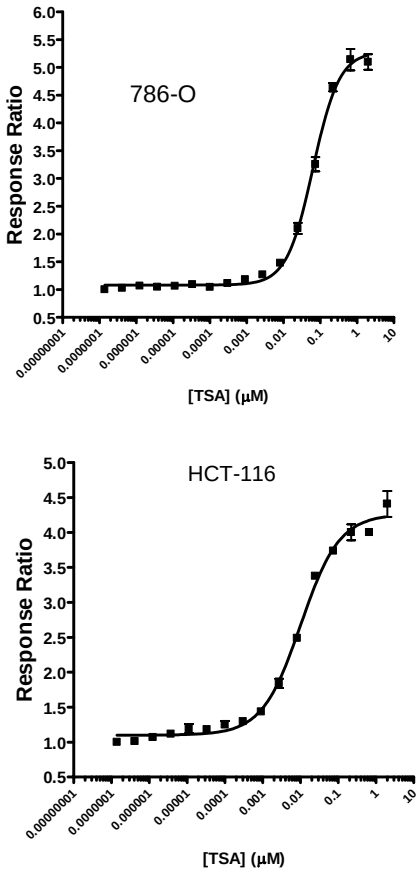
Figure 1h — Localization of GFP-Histone H3

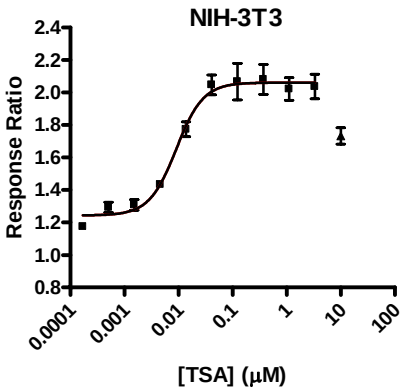


Hoechst GFP-Histone H3

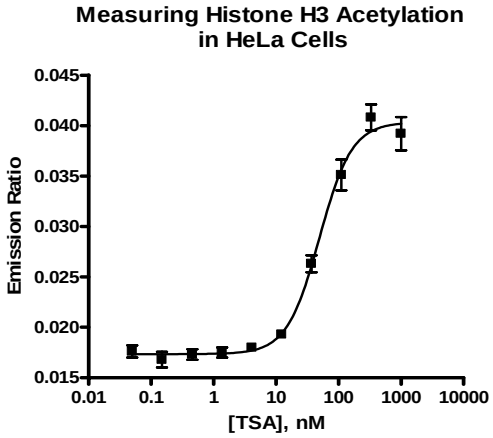
Colocalization of GFP-Histone H3 with DNA in the nucleus. U-2 OS cells were transduced with 10% (v/v) BacMam GFP-Histone H3 reagent for 20 hours prior to Hoechst staining and imaging.

Figure 2a — Alternate cell backgrounds tested





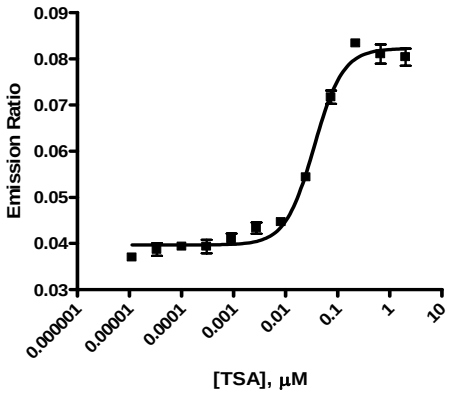
Measurement of Histone H3 acetylation using the BacMam Histone H3 [acLys9] Cellular Assay. Cells were seeded and simultaneously transduced overnight in a T75 flask using 25% (v/v) BacMam Histone H3 Reagent in combination with 0.5 µM Trichostatin A. After 24 hours transduction, cells were harvested, resuspended in assay medium, and then applied to wells of a 384-well plate at a cell density of ~10,000 cells per well. Following an additional 24 hour incubation, cells were treated for 3 hours with a serial dilution of Trichostatin A. Cells were lysed by adding fully-supplemented 6X lysis buffer containing Tb-anti-Histone H3 [acLys9] Antibody and equilibrated for 3 hours at room temperature before TR-FRET was measured on a BMG Labtech Pherastar plate reader.



Measurement of Histone H3 acetylation using the BacMam Histone H3 [acLys9] Cellular Assay. HeLa cells were harvested, resuspended in assay medium and mixed with BacMam Histone H3 Reagent to achieve 30% virus (v/v) concentration. The mix was then applied to wells of a 384-well plate, yielding a cell density of ~10,000 cells per well. Following a 24 hour incubation, cells were treated for 3 hours with serial dilutions of Trichostatin A, SAHA, or HDAC inhibitor III. Cells were lysed by adding fully-supplemented 6X lysis buffer containing Tb-anti-Histone H3 [acLys9] Antibody and equilibrated for 3 hours at room temperature before TR-FRET was measured on a BMG Labtech Pherastar plate reader.

Representative Data for BacMam Histone H3 [AcLys9] Cellular Assay

Measuring Histone H3 Acetylation: Primary Human Dermal Fibroblasts



Measurement of Histone H3 acetylation using the BacMam Histone H3 [acLys9] Cellular Assay. Cryopreserved human neonatal dermal fibroblasts (HDFn) were thawed, resuspended in assay medium and mixed with BacMam Histone H3 Reagent to achieve the indicated virus (v/v) concentrations. The mixes were then applied to wells of a 384-well plate, yielding a cell density of ~5,000 cells per well. Following a 24 hour incubation, cells were treated for 3 hours with a serial dilution of Trichostatin A. Cells were lysed by adding fully-supplemented 6X lysis buffer containing Tb-anti-Histone H3 [acLys9] Antibody and equilibrated for 3 hours at room temperature before TR-FRET was measured on a BMG Labtech Pherastar plate reader.

General References:

- Carlson, C. B.; Mashock, M.J.; Bi, K., BacMam-enabled LanthaScreen cellular assays for PI3K/Akt pathway compound profiling in disease-relevant cell backgrounds. *J. Biomol. Screen* **2010**, *15*, 327-334.
- Robers, M. B.; Horton, R. A.; Bercher, M. R.; Vogel, K. W.; Machleidt, T., High-throughput cellular assays for regulated post-translational modifications. *Anal. Biochem*. **2008**, *372*, 189–197.
- Huwiler, K.G.; Machleidt, T.; Chase, L.; Hanson, B.; Robers, M. B., Characterization of serotonin 5-hydroxytryptamine-1A receptor activation using a phosphor-extracellular-signal regulated kinase 2 sensor. *Anal. Biochem*. **2009**, *393*, 95-104.
- Kost, T. A.; Condreay, J. P.; Ames, R. S.; Rees, S.; Romanos, M. A., Implementation of BacMam virus gene delivery technology in a drug discovery setting. *Drug Disc. Today* **2007**, *12*, 396-403.