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BacMam Histone H3K27me3 Cellular Assay User Guide

Catalog no. A14159 **Shipping:** Varies **Storage:** Varies

Protocol part no. A14159PPS (MAN0005023) **Rev. date:** 17 August 2011

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Kit Contents and Handling

Kit Components	Part no.	Amount	Storage	Handling
LanthaScreen® Tb-anti-Histone H3K27me3 Antibody	A14166	5 µg	–20°C	• Protect from light • Avoid multiple freeze/thaw cycles
LanthaScreen® 6X Cellular Assay Lysis Buffer	A12891	6 mL	4°C	On the day of assay, supplement with protease inhibitor cocktail* and antibody
BacMam Histone H3 Reagent	A12894	25 mL	4°C	• DO NOT FREEZE • Use sterile technique • Avoid extended exposure to ambient room light
Instrument Control Terbium TR-FRET Low Instrument Control, 1 mL High Instrument Control, 1 mL	A14138	1 kit	4°C	Protect from light (do not vortex)

*See **Materials Required but Not Provided**, page 3.

Overview

BacMam Cellular Assays use the BacMam gene delivery system in conjunction with LanthaScreen® Cellular Assays to measure post-translational modifications of a target substrate. The combination of the two technologies provides a fast, convenient, and robust method for interrogating specific signal transduction events in a cell background of choice.

LanthaScreen® Cellular Assay (Terbium-based TR-FRET detection)

LanthaScreen® Cellular Assays are HTS-compatible immunoassays that are used for interrogating target-specific post-translational modifications in a cell-based format. Target proteins are expressed as fusions with the green fluorescent protein (GFP) in living cells, and modification-specific antibodies labeled with Terbium (Tb) are used to detect post-translational modifications of the target protein in a time-resolved fluorescence resonance energy transfer (TR-FRET) format.

The use of GFP as a FRET acceptor circumvents the need for complex antigen-capturing reagents, thereby providing a high-throughput alternative to commonly used analytical methods such as Western blot and ELISA.

For more information, visit **www.invitrogen.com/lanthascreencellular**.

BacMam Technology

While GFP-Histone H3 can be delivered to cells via multiple methods (e.g., stable cell line generation, transient transfection, electroporation, retroviral transduction), BacMam technology is a convenient approach that uses a modified baculovirus to efficiently deliver and transiently express genes (in this case, GFP-Histone H3) in mammalian cells. BacMam viruses are non-replicating in mammalian cells, rendering them safe as research reagents.

This technology has several advantages over traditional transient methods for heterologous gene expression, including:

- High transduction efficiency across a broad range of cell types, including primary and stem cells
- Little-to-no observable cytopathic effects
- Reproducible and titratable target gene expression
- Compatibility with simultaneous delivery of multiple genes

For more information on BacMam, visit **www.invitrogen.com/bacmam**.

Workflow for the BacMam Histone H3K27me3 Cellular Assay

Day 1: Cells are transduced with BacMam GFP-Histone H3 reagent for 20–24 hours, resulting in expression of GFP-Histone H3 fusion protein in the nucleus that can be visualized by fluorescence microscopy.

Day 2: Cells are harvested and plated onto assay plate. Cells are left untreated or treated with compounds (e.g., EZH2 inhibitors) for desired time-point (e.g., 20 to 24 hrs).

Day 3: Cells are lysed in the presence of a Tb-labeled anti-Histone H3K27me3 specific antibody, and the level of K27 tri-methylation on the GFP-Histone H3 is measured on a TR-FRET-compatible plate reader. Little or no TR-FRET generally indicates little or no modification, while high TR-FRET indicates high K27me3 level.

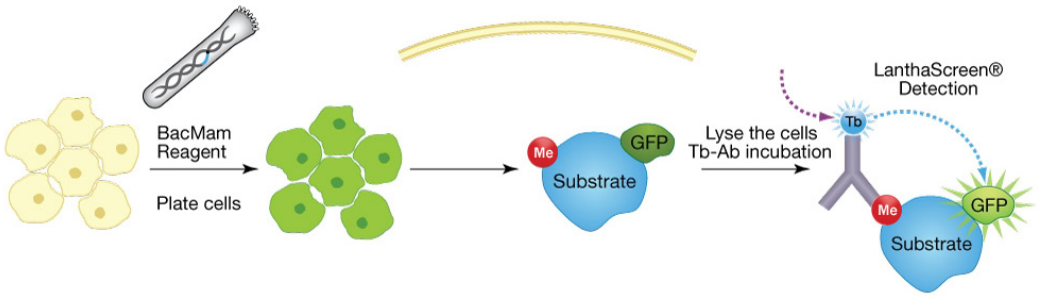

Figure 1 Representative Assay Workflow. Cells are mixed with BacMam Reagent encoding GFP-tagged Histone H3 protein and plated in a 6-well format. 20–24 hours post-transduction, cells are harvested and plated onto a 384-well assay plate. Cells are left untreated or treated with compound for additional 20 to 24 hours. Cells are lysed in the presence of a terbium-anti-Histone H3K27me3 antibody and TR-FRET is detected using a fluorescence microplate reader with standard TR-FRET settings.

Before Starting

Materials Required but Not Provided

Materials	Recommended Source	Part no.
Cell Line of Interest (visit **www.invitrogen.com/bacmam** for more information regarding BacMam compatible cell types)	Various	Various
Assay Medium (commonly used growth media are compatible with the terbium TR-FRET readout; where possible, avoid media containing phenol-red because its presence in the assay can interfere with the TR-FRET signal leading to reductions in assay performance)	Various	Various
Protease Inhibitor Cocktail	Sigma	P8340
Assay Plates (white opaque plates) Tissue culture-treated 384-well assay plates	Corning	3570
Fluorescence plate reader with top-read and TR-FRET capability	**www.invitrogen.com/instrumentsetup**	
Optional: Clear-bottom, tissue culture-treated 384-well assay plates setup in parallel to the TR-FRET assay plates for visualization of GFP-Histone H3 expression	Corning	3712

HeLa Cell Culture Reagents (optional, if using HeLa cells)

HeLa cells transduce well with BacMam viruses, and we recommend their use as a control cell line for detecting H3K27me3.

Media/Reagents	Recommended Source	Part no
HeLa cells	ATCC	CCL-2
Dulbecco's Modified Eagle Medium (D-MEM), liquid (with GlutaMAX™-I)	Invitrogen	10569-010
Fetal Bovine Serum (dialyzed)	Invitrogen	26400-036
Nonessential amino acids (NEAA)	Invitrogen	11140-050
Penicillin/Streptomycin (antibiotic)	Invitrogen	15140-122
HEPES Buffer Solution (1 M)	Invitrogen	15630-080
Dulbecco's Phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{2+}	Invitrogen	14190-136
Trypsin/EDTA	Invitrogen	25300-062
BacMam Enhancer Solution (1000X)	Invitrogen	PV5835
6-well cell culture treated plates	Corning	353046

Guidelines for Optimizing BacMam Histone H3K27me3 Cellular Assays

First-time Terbium TR-FRET users

Prior to setting up an assay, we strongly recommend that you check your plate reader setup for terbium-based TR-FRET detection using the Terbium TR-FRET Instrument Controls (provided in this kit) as outlined on page 8. For more information about your specific instrument and to purchase filters, visit **www.invitrogen.com/instrumentsetup**. For technical assistance, contact Drug Discovery Technical Support at **drugdiscoverytech@lifetech.com** or call 760-603-7200, option 3, extension 40266).

Working with BacMam Histone H3 reagent

- For first time users of BacMam Reagent, we recommend including a control cell line which transduces exceptionally well, such as U-2 OS (ATCC® number: HTB-96) or HeLa (see protocol on page 5).
- Since assay performance depends upon effective expression of GFP-Histone H3, we strongly recommend performing a titration of the BacMam GFP-Histone H3 Reagent to determine the optimal percentage of virus (v/v) for the transduction in your cell background of interest. For initial testing of BacMam GFP-Histone H3 in a given cell-type, we recommend testing a final of ~30%, 20%, 10%, 3%, 1%, and 0.3% (v/v) dilutions. Select the lowest percentage of BacMam Reagent that yields the largest assay window. See **example below**.

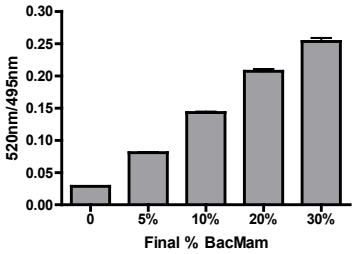

Figure 2. Detection of Histone H3K27me3 in HeLa cells transduced with various concentrations of BacMam Histone H3 Reagent.

- While many cell types such as MCF7, T47D and MDA-MB-231 can be transduced efficiently using the detailed protocol described here for HeLa, some challenging cell types (e.g., CHO or MEF) may require alternative protocols as described in the **Appendix** (page 9). A shorter transduction protocol for easy-to-transduce cells such as U-2 OS is also included in the **Appendix** (page 11).
- For best results, use healthy, well-maintained cells when performing BacMam transductions. Note that for many cell-types, cryopreserved cells can also be transduced immediately following thaw.
- To minimize day-to-day variability, be sure to use the same growth conditions (e.g., similar harvest density, similar passage numbers).
- BacMam Enhancer (Cat. no. PV5835, supplied at 1,000X) can improve the transduction efficiency of difficult-to-transduce cell lines (e.g., see page 5 for HeLa and Appendix: Alternate Transduction Protocol A, page 9). When using the Enhancer, we recommend testing at least two concentrations of it (e.g., 0.5X and 1X) in comparison to a control without Enhancer to identify the best concentration for your cell-type of interest. To minimize the effect of the Enhancer solution on your assay of interest, we recommend removing the Enhancer 20 to 24 hours prior to the LanthaScreen® assay.

Important assay parameters for optimization

- Confluence of cells at harvest for assay set-up may impact results, such as the assay window.
- Cell plating density (i.e., cell number per well in the assay plate)
- Expression level of GFP-fusion protein (i.e., titration of BacMam GFP-Histone H3 reagent)
- Compound concentrations and/or treatment times
- Antibody equilibration time

Application Note

Visit **www.lifetechnologies.com** and search for A14159 to download the application note for the BacMam Histone H3K27me3 Cellular Assay. The application note is located under the "How to Use" tab on the product page, and contains more information about H3K27me3 assay optimization and applications.

Assay Protocol

In the following protocol, cells are incubated with BacMam GFP-Histone H3 Reagent for two 20–24-hour incubation periods. Two alternative transduction protocols are provided in the **Appendix** on pages 9 and 11.

The cell harvesting and plating densities, growth medium, and assay medium must be optimized for your particular cell line(s). The following protocol was developed for HeLa cells. It may be applied to many other cell types such as MBA-MD-231, MCF7 and T47D, although conditions may need to be optimized for different cell types.

Quick Reference Protocol Example (HeLa Cells): 384-well Assay Plate Format

This quick reference protocol is designed for experienced users using HeLa cells, with testing performed in the presence of various concentrations of BacMam Histone H3 reagent. Conditions may need to be optimized for different cell types. For a detailed protocol, see page 6.

		Nontransduced Control Wells	Transduced Control Wells	Test Compound Wells
BacMam Transduction	**Step 1** Grow, harvest, and plate cells onto a 6-well plate	• Grow cells in Growth Medium* to 60–90% confluence (~0.2 × 10^5 to 0.8 × 10^5 cells/cm^2). • Prepare Growth Medium containing 0.75 X BacMam Enhancer Solution by adding 7.5 µL of the 1,000X Enhancer Solution to 10 mL Growth Medium. • Harvest cells and resuspend in Growth Medium containing the BacMam Enhancer Solution at 5 × 10^5 cells/mL. • Plate 2 mL (~1 × 10^6 cells) cell suspension onto each well of a 6-well plate.		
	Step 2 Transduction	Add 1 mL/well Growth Medium only	Add BacMam GFP-Histone H3 reagent to each well so that the final virus concentration is 30% (1 mL BacMam reagent), or 20% (0.6 mL BacMam reagent + 0.4 mL growth medium) and so on.	
	Step 3 Incubate Cells/BacMam	Incubate the plate at 37°C/5% CO_2 for 20–24 hours (allows for GFP-Histone H3 expression)		
LanthaScreen® Histone H3K27me3 Assay	**Step 4 (Optional)** GFP Imaging	If desired, observe and image GFP-Histone H3 expression under a fluorescence microscope using standard FITC filter sets		
	Step 5 Harvest and plate cells onto a 384-well plate	• Harvest cells and resuspend in Growth Medium at 5 × 10^5 cells/mL. • Plate 20 µL/well (~0.1 × 10^5 cells) onto a 384-well assay plate. • Quick spin the plate at 30 × *g* for 1 minute (if performing the experiment manually)		
	Step 6 Compound Treatment	Add 5 µL/well of Growth Medium	Add 5 µL/well of Growth Medium	5 µL/well of 5X Compound in Growth Medium
	Step 7 Incubate Cells	• Quick spin the plate at 30 × *g* for 1 minute (if performing the experiment manually) • Incubate the plate at 37°C/5% CO_2 for additional 20 to 24 hours		
	Step 7 Prepare Complete 6X Lysis Buffer	For each 1 mL of 6X Lysis Buffer, add 30 µL 100X protease inhibitor cocktail, and LanthaScreen® Tb-anti-Histone H3K27me3 Antibody to 12 nM. Scale volume needed to the number of wells × 5 µL/well × 1.2 to ensure extra buffer.		
	Step 8 Add Lysis Buffer (including Tb-Ab)	• Add 5 µL/ well of Complete 6X Lysis Buffer (including Tb-Ab and protease inhibitor) • Quick spin the plate at 30 × *g* for 1 minute (if performing the experiment manually) • Incubate plate for ~2 to 3 hours at room temperature in the dark		
	Step 9 Read Plate and Analyze Data	See **Terbium TR-FRET Detection** on page 8		

***Growth Medium for HeLa Cells:** DMEM Medium supplemented with 10% dFBS, 0.1 mM NEAA, 10 mM HEPES, and 100 U/mL Penicillin 100 µg/mL Streptomycin

Detailed Protocol

For first-time Terbium TR-FRET users, we strongly recommend testing your instrument setup using the set of HIGH and LOW instrument controls provided in the kit prior to setting up the assay. See **Terbium TR-FRET Detection** on page 8 for details.

In this protocol, cells are incubated with virus at the time of adhering to the tissue-culture plate, usually 20–24 hours prior to re-plating onto the assay plate.

Day 1: BacMam Transduction

1. Begin with HeLa cells in Growth Medium* at 60–90% confluency (~0.2 × 10^5 to 0.8 × 10^5 cells/cm^2). Confluency of the cells may impact results, such as the assay window.
2. Trypsinize to harvest the cells as recommended by the cell line manufacturer.
3. Prepare Growth Medium containing 0.75 X BacMam Enhancer Solution by adding 7.5 µL of the 1,000X Enhancer Solution to 10 mL Growth Medium.
4. Resuspend cells in Growth Medium containing the BacMam Enhancer Solution at 5 × 10^5 cells/mL.
5. Plate 2 mL (~1 × 10^6 cells) cell suspension onto each well of a 6-well plate.
6. Immediately after seeding the cells, add the desired amount of BacMam Reagent to the cells. For initial optimization, we recommend testing ~30%, 20%. 10%, 3%, 1%, and 0.3% v/v dilutions of BacMam Reagent (e.g., for testing in 6-well plate format containing ~2 mL cell suspension per well, add 300 µL of BacMam Reagent for an ~10% v/v dilution and then 700 µL of growth medium so that the final total volume is 3 mL/well).
7. Place cells in a humidified 37°C/5% CO_2 incubator for 20-24 hours to allow for the transduction and expression of the GFP fusion protein.

Day 2: Cell Harvest and Plating onto a 384-well Assay Plate

1. *Optional:* Analyze GFP expression levels by fluorescence microscopy using standard FITC filter sets.
2. Harvest the transduced cells and be careful not to over-trypsinize the cells as this can result in poor viability and a decreased assay window.
3. Resuspend the harvested cells in Growth Medium with serum to inactivate the trypsin.
4. Count cells and then centrifuge the cells at 200 × g for 5 minutes. Aspirate the Growth Medium and resuspend the cell pellet with Growth Medium (without the BacMam Enhancer solution) at a density of 0.5 × 10^6 cells/mL.

Tip: The number of cells per well can affect the assay window and should be optimized for your cell background of interest. We recommend starting with 7,500–20,000 cells per well seeded in 20 µL of Growth Medium for 384-well format.

5. Plate 20 µL/well of cells onto a 384-well assay plate.
6. *Optional:* Plate cells in a parallel clear-bottom plate for image analysis of GFP expression.

Tip: If the experiment is performed manually, we recommend quickly spinning the assay plates at 30 × *g* for 1 minute after plating cells.

7. If you are testing compound effect on the H3K27me3 level, add 5 µL/well of 5X Compound in Growth Medium to the Compound Treated Wells and 5 µL/well of Growth Medium to the no-compound wells. If the compound is dissolved in DMSO, make sure the amount of DMSO per well remain consistent in all the wells.

Tip: If the experiment is performed manually, we recommend quickly spinning the assay plates at 30 × *g* for 1 minute after compound addition.

8. Incubate plates for 20–24 hours in a humidified incubator at 37°C/5% CO_2.

Day 3. LanthaScreen® Cellular Assay

1. *Optional:* 20 to 24 hours later, analyze GFP expression levels in the clear-bottom plate by fluorescence microscopy using standard FITC filter sets.
2. Prepare **Complete 6X lysis buffer** by adding protease inhibitor cocktail at a 1:33 dilution (e.g., 30 µL of 100X stock protease inhibitor per 1,000 µL of LanthaScreen® 6X Cellular Assay Lysis Buffer) and adding Tb-anti-Histone H3K27me3 antibody at a concentration of 12 nM. Mix by pipetting up and down gently. Store on ice until use (prepare fresh on day of assay).

Note: We recommend scaling the volume of Complete 6X Lysis Buffer as follows:

Number of assay wells to be lysed × 5 µL/well × 1.2 scaling factor

(Scaling factor ensures some extra Lysis Buffer to offset any loss to pipette dispensing tips, dead volumes, etc.; the scaling factor may need to be adjusted based on your dispensing setup).

3. Add 5 µL/well of Complete 6X Lysis Buffer. Cover the plate.
4. *Optional:* Separately adding 20 µL/well in 384-well format of the HIGH and LOW instrument controls to blank assay plate wells. We recommend plating a minimum of 3 replicates of each control.

Tip: If the experiment is performed manually, we recommend quickly spinning the assay plates at 30 × *g* for 1 minute after adding the lysis buffer.

5. Incubate the covered plate at room temperature in the dark for ~2–3 hours or another desired antibody equilibration time. The equilibration time can be optimized for your cell line of interest (typically 60 minutes to several hours).

Note: The assay plate may also be stored at 4°C overnight prior to reading. Allow the plate to warm to room temperature prior to reading. (Longer storage time at 4°C is also possible if evaporation is minimized).

6. Proceed to reading the plate, as described in the next section.

Terbium TR-FRET Detection

Instruments and Filters

Terbium TR-FRET-based cellular assays can be performed on a variety of plate readers, such as the PE Envision. For more information on your particular instrument, refer to **www.invitrogen.com/instrumentsetup** or contact Life Technologies Discovery Sciences technical support (**drugdiscoverytech@lifetech.com** or 760-603-7200, option 3, extension 40266).

Reading the Assay Plate and Data Analysis

All measurements should be taken at room temperature from the top of the wells, **with the plate lid or plate seal removed**.

1. Let the assay plate warm to room temperature before reading, if necessary.
2. Set the fluorescence plate reader to top/time-resolved read mode. Allow the lamp in the plate reader to warm up for at least 10 minutes before taking measurements.
3. Remove the lid or plate seal and read the plate using the appropriate filters. Note that filter bandwidths are critical and cannot be approximated. For instrument-specific setup details, refer to **www.invitrogen.com/instrumentsetup**.

Note: We do not recommend using monochromator-based instruments without adjustable bandwidth, because the sensitivity of these instruments is not sufficient to adequately detect the TR-FRET signal.

4. Calculate the acceptor/donor Emission Ratio (520 nm for acceptor and 490 nm or 495 nm for donor) for each well, by dividing the acceptor emission values by the donor emission values. Do **not** average the 520 nm and 490 nm or 495 nm reading and then take the ratio.
5. *Optional:* Convert the data to Assay Window by dividing each emission ratio value by the value from Non-transduced control wells (or cells not expressing any GFP-Histone H3).

Testing Terbium TR-FRET Detection Using the Instrument Controls

To test your instrument set-up for performing terbium-based TR-FRET cellular assays:

1. Separately add 20 µL/well (for 384-well format) of the HIGH and LOW instrument controls to blank assay plate wells. We recommend plating a minimum of 3 replicates of each control.
2. Read the plate, as described in the above section.
3. Calculate the acceptor/donor Emission Ratio (520 nm/495 nm or 490nm) for each well, by dividing the acceptor emission values (520 nm) by the donor emission values (495 nm or 490 nm).
4. Determine the HIGH/LOW ratio by dividing the average Emission Ratio from the HIGH control wells by the average Emission ratio from the LOW control wells. This value should be between 2–4 depending on the specific plate reader used.

Appendix

The following alternative transduction protocols may be used with difficult-to-transduce cell lines (below) or very easy-to-transduce cells such as U-2 OS (page 11).

In short, the alternative protocol for difficult-to-transduce cells requires that the cells be allowed to adhere to the tissue culture flasks prior to the transduction with the BacMam Reagent (the longest protocol, but generally allows for higher transduction efficiency for difficult cell types). The alternative protocol for easy-to-transduce cells in a 384-well plate requires that cells are incubated with virus at the time of plating onto an assay plate.

Alternative Transduction Protocol (for difficult-to-transduce cells)

In this protocol, cells are allowed to adhere to the tissue-culture flasks before transduction with BacMam Reagent.

Day 1: Adhere Cells

1. Begin with cells grown to complete confluence in normal tissue-culture flasks. Confluency of the cells may impact results, such as the assay window.
2. Trypsinize and harvest adherent cells as recommended by the cell line manufacturer.
3. Plate the desired number of cells in Growth Medium and allow them to adhere (typically 16–24 hours).

Tip: For many cell types (with a doubling time of approximately 24 hours), a seeding density of approximately 2×10^4–4×10^4 cells/cm^2 will result in 50–80% confluency 24 hours after seeding. This has proven optimal for transducing cell lines such as CHO. It may be necessary to optimize the cell density for specific cell backgrounds.

Day 2: Transduce Cells

1. Determine the volume of BacMam Reagent necessary to cover the adhered cells in the tissue culture flask. We recommend ~1 mL of BacMam solution (diluted as described in the next step) for every 10 cm^2 of flask surface area.
2. Prepare a dilution of BacMam Reagent (v/v) in Dulbecco's Phosphate Buffered Saline (dPBS) containing Ca^{2+} and Mg^{2+} (Cat. no. 14040-133). We recommend testing a range of v/v dilutions of BacMam Reagent and using 30%, 20% 10%, 3%, and 1% (v/v) as a starting point (e.g., add 100 µL of BacMam Reagent to 900 µL of dPBS for a 10% v/v dilution).
3. Gently wash the cells once with dPBS containing Ca^{2+} and Mg^{2+}.
4. Remove dPBS from Step 3, and gently add the solution of the diluted BacMam Reagent from Step 2 to the cells. Incubate the cells at room temperature (20 –25ºC) for 2–4 hours, protected from light.
5. Aspirate the transduction solution from the cell culture dish.
6. Add an appropriate volume of complete cell culture growth medium or growth medium containing the BacMam Enhancer Solution.
7. BacMam Enhancer Solution (Cat. no. PV5835) can improve the transduction efficiency with difficult-to-transduce cell lines. When using the Enhancer, we recommend testing at least two concentrations of it (e.g., 0.5X and 1X) in comparison to a control without Enhancer to identify the best concentration for your cell-type (i.e., the concentration that yields good GFP-Histone H3 expression with little or no detectable Enhancer-associated toxicity).
8. Incubate cells for 20 –24 hours in a humidified 37°C/5% CO_2 incubator.

Day 3: Harvest & Plate Cells

1. *Optional:* Analyze GFP expression levels by fluorescence microscopy using standard FITC filter sets.
2. Harvest the transduced cells and be careful not to over-trypsinize the cells as this can result in poor viability and a decreased assay window.
3. Resuspend the harvested cells in Growth Medium with serum to inactivate the trypsin. Centrifuge the cells at 200 × *g* for 5 minutes. Aspirate the Growth Medium, resuspend the cell pellet in Assay Medium (could be the same as Growth Medium), and plate the cells.

Tip: The number of cells per well can affect the assay window and should be optimized. We recommend starting with 7,500–20,000 cells per well seeded in a 384-well format.

4. Plate 20 µL/well of cells onto a 384-well assay plate.
5. *Optional:* Plate cells in a parallel clear-bottom plate for image analysis of GFP expression.

Tip: If the experiment is performed manually, we recommend quickly spinning the assay plates at 30 × *g* for 1 minute after plating cells.

6. If you are testing compound effect on the H3K27me3 level, add 5 µL/well of 5X Compound in Growth Medium to the Compound Treated Wells and 5 µL/well of Growth Medium to the no-compound wells. If the compound is dissolved in DMSO, make sure the amount of DMSO per well remain consistent in all the wells.

Tip: If the experiment is performed manually, we recommend quickly spinning the assay plates at 30 × *g* for 1 minute after compound addition.

7. Incubate plates for 20–24 hours in a humidified incubator at 37°C/5% CO_2.

Day 4. LanthaScreen® Cellular Assay

1. Proceed to the **LanthaScreen® Cellular Assay** on page 7.

Alternative Transduction Protocol in a 384-well Plate (for easy-to-transduce cells, such as U-2 OS)

Day 1. Cell Preparation and BacMam Transduction

1. Begin with healthy cell cultures grown under normal Growth Medium conditions. Confluence of cells may impact results, such as the assay window.

Note: To minimize day-to-day variability, use consistent growth conditions (e.g., similar harvest density, similar passage numbers).

Note: For many cell-types, cryopreserved cells can be transduced immediately following thaw to save culturing time.

2. Harvest cells and resuspend them in Growth Medium or nutrient-rich Assay Medium using the appropriate conditions for your particular cell line. (For U-2 OS cells, resuspend at ~7.5 × 10^5 cells/mL).

Note: Add BacMam Histone H3 Reagent to the cells. A typical final concentration of BacMam Reagent is 5–30% (i.e., volume of virus : volume of cell media). Mix gently by inversion.

Note: We recommend testing a range of v/v dilutions of BacMam Reagent to optimize for GFP-Histone H3 expression. For U-2 OS cells, we recommend preparing four 3-fold serial dilutions of the BacMam Reagent in Growth Medium (e.g., add 100 µL BacMam into 200 µL Growth Medium, mix, then dilute 100 µL into 200 µL, repeat; scale the amount to need). Then mix 400 µL of U-2 OS cells at ~7.5 × 10^5 cells/mL with 200 µL of undiluted virus and each of the four BacMam Reagent dilutions. This should yield a final U-2 OS cell concentration of ~5.0 × 10^5 cells/mL and final BacMam GFP-Histone H3 test concentrations of ~33%, 11%, 3.3%, 1% and 0.3% (v/v).

3. Transfer 20 µL/well cells/BacMam mixture onto a 384-well assay plate. The number of cells per well may need to be optimized for your cell type of interest (e.g., seed U-2 OS cells at 10,000 cells/well in a 384-well format).

Tip: If the experiment is performed manually, we recommend quickly spinning the assay plates at 100 × *g* for 1 minute after plating cells.

4. If you are testing compound effect on the H3K27me3 level, add 5 µL/well of 5X Compound in Growth Medium to the Compound Treated Wells and 5 µL/well of Growth Medium to the no-compound wells. If the compound is dissolved in DMSO, make sure the amount of DMSO per well remain consistent in all the wells.

Tip: If the experiment is performed manually, we recommend quickly spinning the assay plates at 30 × *g* for 1 minute after compound addition.

5. Incubate plates for 20–24 hours in a humidified incubator at 37°C/5% CO_2.

Day 2. LanthaScreen® Cellular Assay

1. Proceed to the **LanthaScreen® Cellular Assay**, page 7.

Troubleshooting Guide

Observation	Potential Solutions
Weak/no expression of GFP-fusion in the cell line of interest in a clear-bottom assay plate.	Confirm that your fluorescence microscope is configured appropriately for detection of GFP/FITC.
	Perform a virus titration to find the optimal virus concentration for your cell background.
	Confirm that no contamination of the BacMam Reagent has occurred. Contaminated reagent looks cloudy or contains chunks of mold.
	For first-time users, we recommend the standard transduction protocol using HeLa cells.
	If the standard protocol works for HeLa cells but not for your cells, try one of the Alternative Transduction Protocols in the Appendix (pages 9 and 11, respectively).
>50% expression of GFP-fusion is observed, but weak/no detectable TR-FRET signal over background is detected.	Confirm that the fluorescence plate reader is configured appropriately for Terbium TR-FRET detection. Filter bandwidth requirements are exact. For more information about your specific instrument and to purchase filters, visit **www.invitrogen.com/instrumentsetup**. Contact Invitrogen Discovery Sciences Technical Support at 760-603-7200, option 3, extension 40266 or **drugdiscoverytech@lifetech.com** for more information.
	For first-time users, we recommend following the standard transduction protocol using HeLa cells (page 5).
	Perform a cell density experiment to find out the optimal cell harvesting density for your cell line of interest.
	Image the cells in clear-bottom microtiter plates. Ensure that cells are adhered to the bottom of the plate and are not expressing very high levels of GFP. Dimly green cells are desirable. Excessive expression of the GFP-fusion may be deleterious to cell health.
Day-to-day fluctuations in assay window are observed.	Be sure to use cells with the same growth conditions (e.g., same harvest density).

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