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Product Information Sheet

BacMam Histone H3K9me2 Cellular Assay

Catalog Number: A14161
Literature Lot Number: V1

Literature Part Number: A14161.PIS (MAN0006204)
Revision date: 12 December 2011

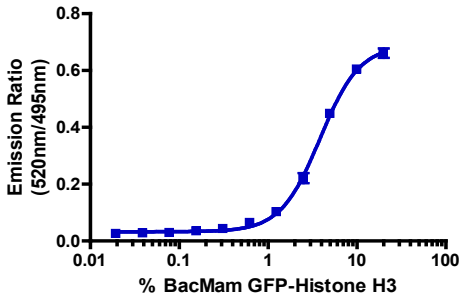
FAST FACTS

For first-time BacMam users, we recommend using cells like U-2 OS following the detailed protocol available online.

Optimal Virus Concentration: We recommend performing a titration of the BacMam Histone H3 Reagent to determine the optimal percentage of virus for transduction in your cell background of interest or when you receive a new lot of virus. Select the lowest percentage of BacMam Reagent that yields the largest assay window (response ratio). See example below.

Component	Part no.	Amount	Storage	Handling
LanthaScreen® Tb-anti-Histone H3K9me2 Antibody	A14160	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 light
Instrument Control Terbium TR-FRET Kit Low Instrument Control, 1 mL High Instrument Control, 1 mL	A14138	1 kit	4°C	• Protect from light (do not vortex)

Titration of BacMam Histone H3 Reagent in U-2 OS cells (Detection of Histone H3K9me2)



Additional Materials Required, but not provided	Source	Part no.
Cell Line of Interest	Various	Various
Protease Inhibitor Cocktail	Sigma	P8340
White tissue culture-treated, 384-well assay plates	Corning	3570
Fluorescence plate reader with top-read and TR-FRET capability	www.lifetechnologies.com/instrumentsetup for details	
Optional: Clear-bottom, tissue-culture treated, 384-well plates	Corning	3712

Detailed Protocols and Additional Assay Performance Data Available

Visit www.lifetechnologies.com and search for A14161 to download the full detailed protocol and application note for this assay. Protocols and application note are located under the "Manuals" tab on the product page.
Application Notes include assay performance under variable experimental conditions.

Technical Support

For additional assistance in running this BacMam-enabled Cellular Assay, please contact our technical support team at drugdiscoverytech@lifetechnologies.com or 760-603-7200 (enter 3 for "know your party's extension", then enter 40266).

Quick Reference Protocol for Transduction and LanthaScreen® Cellular Assay using U-2 OS Cells

This quick reference protocol is designed for experienced users using U-2 OS 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 the protocol on our web site.

<table>
<tr><th></th><th></th><th>Non-transduced Wells</th><th>Transduced Wells</th></tr>
<tr><td rowspan="3">BacMam Transduction</td><td>Step 1
Grow, harvest and plate cells</td><td colspan="2">• Grow cells in Growth Medium* to 80–95% confluency (~0.6 × 10^5 to 1.0 × 10^5 cells/cm^2).
• Harvest cells and resuspend in Growth Medium at 3.75 × 10^5 cells/mL.
• Plate 20 µL/well of cell suspension (about 7,500 cells/well) onto a 384-well assay plate (and optionally a separate plate with clear-bottom for GFP imaging later).
• Quick spin the plate at 30 × g for 1 minute (if performing the experiment manually).</td></tr>
<tr><td>Step 2**
Add BacMam Reagent</td><td>Add 5 µL/well of Growth Medium.</td><td>Add 5 µL/well of BacMam GFP-Histone H3 reagent (undiluted or diluted with growth medium to result in different concentrations of the BacMam).</td></tr>
<tr><td>Step 3
Incubate Cells/BacMam</td><td colspan="2">• 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 20–24 hours (allows for GFP-Histone H3 expression).</td></tr>
<tr><td rowspan="4">LanthaScreen® Histone H3K9me2 Assay</td><td>Step 4 (Optional)
GFP Imaging</td><td colspan="2">If desired, observe and image GFP-Histone H3 expression under a fluorescence microscope using standard FITC filter sets (if cells/virus were plated on a separate plate with a clear-bottom).</td></tr>
<tr><td>Step 5
Prepare Complete 6X Lysis Buffer</td><td colspan="2">For 1 mL of 6X Lysis Buffer, add 30 µL of 100X protease inhibitor cocktail, and LanthaScreen® Tb-anti-Histone H3K9me2 Antibody to 6 nM.
Scale volume needed to the number of wells × 5 µL/well × 1.2 to ensure extra buffer.</td></tr>
<tr><td>Step 6
Add Lysis Buffer (including Tb-Ab)</td><td colspan="2">• 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.</td></tr>
<tr><td>Step 7
Read Plate and Analyze Data</td><td colspan="2">See Terbium TR-FRET Detection in the detailed protocol online.</td></tr>
</table>

*__Growth Medium__ for U-2 OS Cells: McCoy's 5A Medium supplemented with 10% dFBS, 10 mM HEPES, 0.1 mM NEAA, 1 mM Sodium Pyruvate, and 100 U/mL Penicillin/100 µg/mL Streptomycin

** Once the optimal BacMam concentration is determined, BacMam reagent can be added to the cell suspension in **Step 1** to the optimal concentration (v/v). Cells/virus mixture can then be plated onto the 384-well assay plate at 20 µL/well (7,500 cells/well). For **inhibitory compound treatment**, add 5 µL/well of the 5X compound in Growth Medium and then incubate for 20 to 24 hours prior to **Step 5** and the addition of lysis buffer.

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