Product Information Sheet

BacMam Histone H3 [pSer10] Cellular Assay

Catalog Number: A12898 **Literature Part Number: A12898PIS** (MAN0003207)
Literature Lot Number: V1 Revision date: 13 August 2010

FAST FACTS

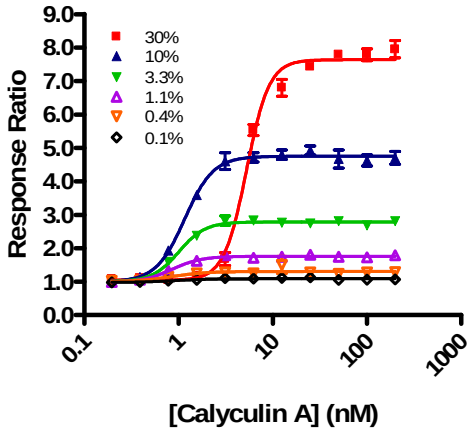
For first-time BacMam users, we recommend using cells like U-2OS (ATCC, HTB-96) which can be transduced exceptionally well.

BacMam Enhancer Solution (PV5835) is **not** required for this assay.

Optimal Virus %: We recommend that you perform a titration of the BacMam Histone H3 Reagent to determine the optimal percentage of virus for your 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.

Kit Components	Amount	Storage	Handling
BacMam Histone H3 Reagent	2 × 25 mL	4°C	• Do not freeze • Minimize exposure to ambient light • Use sterile technique • Aliquot to minimize handling, if necessary
LanthaScreen® Tb-anti-Histone H3 [pSer10] Antibody	10 µg	–20°C	Aliquot if necessary to avoid multiple freeze/thaw cycles
6X LanthaScreen® Cellular Assay Lysis Buffer	6 mL	4°C	Supplement with inhibitors and antibody

Titration of BacMam Histone H3 Reagent in U-2OS cells (Detection of Histone H3 phosphorylation at Ser19 induced by calyculin A)



Additional Materials Required, but not provided	Source	Part #
Positive Control Agonist **We recommend:** Calyculin A (prepare stock solution at 1,000 µM in DMSO)	EMD Biosciences	208851
Cell Line of Interest	Various	Various
DMSO	Fluka	41647
Protease Inhibitor	Sigma	P8340
Phosphatase Inhibitor	Sigma	P0044
White tissue culture-treated, 384-well assay plates	Corning	3570
Fluorescence plate reader with top-read and TR-FRET capability	www.invitrogen.com/instrumentsetup for details	
Optional: Clear-bottom, tissue-culture treated, 384-well plates	Corning	3712

Detailed Protocols and Additional Assay Performance Data Available

Please visit www.invitrogen.com and search for A12898 to download the full protocol and validation packet for this assay. Protocols and validation packets are located under the "How to Use" tab on the product page.
Validation Packets 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@lifetech.com or 760-603-7200, extension 40266.

Quick Reference Protocol for U-2 OS Cells—BacMam Transduction and Agonist Assays

This protocol is designed for experienced users using U-2 OS cells. Conditions may need to be optimized for different cell types. See the online protocol for more detailed information.

		Cell-Free Control Wells	Unstimulated Control Wells	Stimulated Control Wells	Test Compound Wells
BacMam Transduction	**Step 1** Grow, Harvest Cells and Transduce	• Grow cells in Growth Medium* to 70–90% confluency (~0.75 to 1.0×10^5 cells/cm^2). • Harvest, wash cells and resuspend in Assay Medium** at 3.5 × 10^5 cells/mL. • Perform five 3-fold serial dilutions of BacMam reagent in Assay Medium. • Add 0.4 mL undiluted or serial diluted BacMam reagent to 1 mL cells to generate a virus titration range of 0.1% to 30% (v/v) final concentration.			
	Step 2 Plate Cells/virus mixture	Add 20 µL/well Assay Media only	Add 20 µL cells and BacMam mixture per well (~5000 cells/well), and pulse spin the plate		
	Step 3 Incubate Cells	Incubate the plate at 37°C/5% CO_2 for 20–24 hours			
LanthaScreen® Assay	**Step 4** Add media, control agonist (calyculin A), or test compounds	Add 10 µL/well of 0.3% DMSO in Assay Media		Add 10 µL/well of 3X Agonist (for calyculin, 0.3 µM) in Assay Media	Add 10 µL/well of 3X Test Compound in Assay Media
	Step 5 Stimulate Cells	Incubate the plate at 37°C/5% CO_2 for 1 hour			
	Step 6 Prepare Complete 6X Lysis Buffer	To 1 mL of 6X Lysis Buffer, add 30 µL 100X protease inhibitor, 30 µL 100X phosphatase inhibitor, and Tb-anti-Histone H3 [pSer10] Antibody to 12 nM			
	Step 7 Add Lysis Buffer (including Tb-Ab)	Add 6 µL/ well of Complete 6X Lysis Buffer to each well			
	Step 8 Cell Lysis/Assay Equilibration	Incubate plate for ~3 hours at room temperature in the dark			
	Step 9 Read Plate and Analyze Data	See **LanthaScreen® Detection** in the online protocol—Excitation filter: 337 nm (30 nm bandwidth); Emission filters: 490 nm (10 nm bandwidth) and 520 nm (25 nm bandwidth)***			

* **Growth Media:** McCoy's 5A Media (Invitrogen 16600) with 10% dFBS, 10 mM HEPES, 0.1 mM NEAA, 1 mM Sodium Pyruvate, and 100 U/mL Penicillin/ 100 µg/mL Streptomycin

** **Assay Media:** Opti-MEM® I (Invitrogen 11058) with 0.5% cdFBS (or dialyzed FBS), 0.1 mM NEAA, 1 mM Sodium Pyruvate, and 100 U/mL Penicillin/ 100 µg/mL Streptomycin

*** **Instrument Specific Set-up Guides:** describe instrument specific filters, dichroic mirrors and reading parameters, available at www.invitrogen.com/instrumentsetup.