

BacMam Histone H3K27me3 Cellular Assay

Catalog Number: A14159

Literature Part Number: A14159.PIS (MAN0005185)

Literature Lot Number: V1

Revision date: 16 August 2011

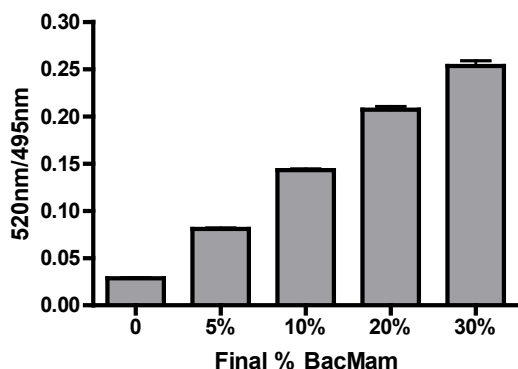
FAST FACTS

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

Optimal Virus %: 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.

Kit Components	Part no.	Amount	Storage	Handling
LanthaScreen® Tb-anti-Histone H3K27me3 Antibody	A14166	5 µg	-20°C	<ul style="list-style-type: none"> Protect from light Avoid multiple freeze/thaw cycles
LanthaScreen® 6X Cellular Assay Lysis Buffer	A12891	6 mL	4°C	<ul style="list-style-type: none"> On the day of assay, supplement with protease inhibitor cocktail and antibody
BacMam Histone H3 Reagent	A12894	25 mL	4°C	<ul style="list-style-type: none"> DO NOT FREEZE Use sterile technique Avoid extended exposure to ambient room light
Instrument Control Terbium TR-FRET kit Low Instrument Control, 1 mL High Instrument Control, 1 mL	A14138	1 kit	4°C	<ul style="list-style-type: none"> Protect from light (do not vortex)

Titration of BacMam Histone H3 Reagent in HeLa cells (Detection of Histone H3K27me3)



Additional Materials Required, but not provided	Recommended Source	Part no.
BacMam Enhancer Solution (1000X)	Life Technologies	PV5835
Cell Line of Interest	Various	Various
Protease Inhibitor	Sigma	P8340
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
6-well cell culture treated plates	Corning	353046

Detailed Protocols and Additional Assay Performance Data Available

Visit www.lifetechnologies.com and search for A14159 to download the full detailed protocol and application note for this assay. Protocols and application note are located under the "How to Use" 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, contact our technical support team at drugdiscoverytech@lifetech.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 HeLa Cells

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 the protocol on our web site.

		Non-transduced Control Wells	Transduced Control Wells	Test Compound Wells
BacMam Transduction	Step 1 Grow, harvest, and plate cells onto a 6-well plate	<ul style="list-style-type: none"> Grow cells in Growth Medium* to 60–90% confluency ($\sim 0.2 \times 10^5$ to 0.8×10^5 cells/cm²). Prepare Growth Medium containing 0.75X BacMam Enhancer Solution by adding 7.5 μL of the 1000X 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 ($\sim 1 \times 10^6$ cells) cell suspension onto each well of a 6-well plate. 		
	Step 2 Transduction	Add 1 mL/well Growth Media 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	<ul style="list-style-type: none"> Harvest cells and resuspend in Growth Medium at 5×10^5 cells/mL. Plate 20 μL/well ($\sim 0.1 \times 10^5$ cells) onto a 384-well assay plate. Quick spin the plate at $30 \times g$ for 1 minute (if performing the experiment manually) 		
	Step 6 Compound Treatment	Add 5 μ L/well of Growth Medium	Add 5 μ L/well of Growth Medium	5 μ L/well of 5X Cmpd in Growth Medium
	Step 7 Incubate Cells	<ul style="list-style-type: none"> Quick spin the plate at $30 \times g$ for 1 minute (if performing the experiment manually) Incubate the plate at 37°C/5% CO₂ for additional 20 to 24 hours 		
	Step 7 Prepare Complete 6X Lysis Buffer	For each 1 mL of 6X Lysis Buffer, add 30 μ L 100X protease inhibitor cocktail, and LanthaScreen® Tb-anti-Histone H3K27me3 Antibody to 12 nM. Scale volume needed to number of wells $\times 5 \mu$ L/well $\times 1.2$ to ensure extra.		
	Step 8 Add Lysis Buffer (including Tb-Ab)	<ul style="list-style-type: none"> Add 5 μL/ well of Complete 6X Lysis Buffer (including Tb-Ab and protease inhibitor) Quick spin the plate at $30 \times g$ 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 7 in the detailed protocol online		

*Growth Media for HeLa Cells: DMEM Media supplemented with 10% dFBS, 0.1 mM NEAA, and 100 U/mL Penicillin/100 μ g/mL Streptomycin

This product is subject to one or more limited use label licenses.

Refer to www.lifetechnologies.com for the corresponding limited use label licenses.

The performance of this product is guaranteed for six months from the date of purchase if stored and handled properly.

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

©2011 Life Technologies Corporation. All rights reserved.

Purchaser Notification

Limited Use Label License: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

This product is sold under license from Columbia University. Rights to use this product are limited to research use only. No other rights are conveyed. Inquiry into the availability of a license to broader rights or the use of this product for commercial purposes should be directed to Columbia Innovation Enterprise, Columbia University, Engineering Terrace-Suite 363, New York, New York 10027.

THIS PRODUCT IS SOLD UNDER PATENT LICENSE FROM MONSANTO FOR RESEARCH PURPOSES ONLY AND NO LICENSE FOR COMMERCIAL USE IS INCLUDED. REQUESTS FOR LICENSES FOR COMMERCIAL MANUFACTURE OR USE SHOULD BE DIRECTED TO DIRECTOR, MONSANTO CORPORATE RESEARCH, 800 N. Lindbergh, St. Louis, Missouri 63167.

Limited Use Label License No. 308: WPRE Element

This product contains the Woodchuck Post-transcriptional Regulatory Element ("WPRE") which is the subject of intellectual property owned by The Salk Institute for Biological Studies, and licensed to Life Technologies Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; and/or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. In addition, any use of WPRE outside of this product or the product's authorized use requires a separate license from the Salk Institute. Life Technologies will not assert a claim against the buyer of infringement of patents owned by Life Technologies and claiming this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product or for a Commercial Purpose. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008, Phone (760) 603-7200. Fax (760) 602-6500, or The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, Attn.: Office of Technology Management, Phone: (858) 453-4100 extension 1275, Fax: (858) 546-8093.

Limited Use Label License No. 332: BacMam Virus Use

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer solely in accordance with the accompanying product literature or manual. Purchase of this product does not convey a license to expand, amplify, or otherwise propagate the provided viral particles or to otherwise modify or alter the virus by any means.

LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

© 2011 Life Technologies Corporation. All rights reserved. Reproduction forbidden without permission.

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

For research use only. Not intended for human or animal therapeutic or diagnostic use.