Product Information Sheet

BacMam p53 [AcLys382] Cellular Assay

Catalog Number: A12901 **Literature Part Number: A12901PIS** (MAN0003209)

Literature Lot Number: V1 Revision date: 13 August 2010

FAST FACTS

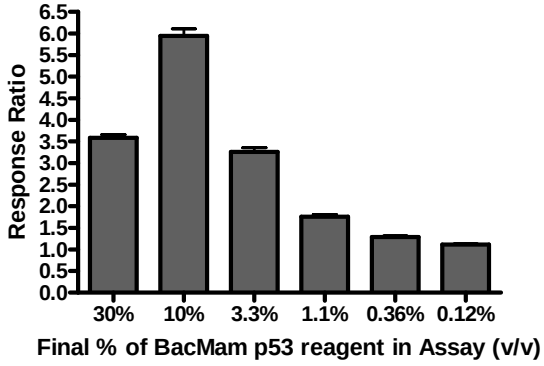
For first-time BacMam users, we recommend using cells like U-2OS (ATCC, HTB-96) which can be transduced exceptionally well.

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

Optimal Virus %: We recommend that you perform a titration of the BacMam p53 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 p53 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-p53 [AcLys382] 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 p53 Reagent in U-2 OS cells (Detection of p53 acetylation at Lys382 induced by 1 µM TSA and 1 µM EX527)



Additional Materials Required, but not provided	Source	Part #
Positive Control Agonist (HDAC/SIRT inhibitors) **We recommend:** Trichostatin A (TSA) (prepare stock solution at 1 mM in DMSO) and EX527 (prepare stock solution at 10 mM in DMSO)	Sigma (TSA)	T8582
	Tocris (EX527)	2780
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 A12895 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-2OS 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 Compound incubation	**Step 1** Grow, Harvest and Transduce Cells	• Grow cells in Growth Medium* to 60–90% confluency (~0.6 to 1.2×10^5 cells/cm^2). • Harvest, wash cells and resuspend in Assay Medium** at 7 × 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 Prepare control and test compound plate	Add 10 µL/well of 0.6% DMSO in Assay Media		Add 10 µL/well of 3X TSA (3 µM) and 3X EX527 (30 µM) in Assay Media	Add 10 µL/well of 3X Test Compound in Assay Media
	Step 3 Plate Cells/virus mixture	Add 20 µL/well Assay Media only	Add 20 µL cells and BacMam mixture per well (~10,000 cells/well), and briefly pulse spin the plate		
	Step 4 Incubate Cells	Incubate the plate at 37°C/5% CO_2 for 16–20 hours			
LanthaScreen® Assay	**Step 5** Prepare Complete 6X Lysis Buffer	To 1 mL of 6X Lysis Buffer, add 30 µL 100X protease inhibitor, 30 µL 100X phosphatase inhibitor, and LanthaScreen® Tb-anti-p53 [AcLys382] Antibody to 12 nM			
	Step 6 Add Lysis Buffer (including Tb-Ab)	Add 6 µL/ well of Complete 6X Lysis Buffer to each well			
	Step 7 Cell Lysis/Assay Equilibration	Incubate plate for ~3 hours at room temperature in the dark			
	Step 8 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) 0.1% 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.

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