Corning® HYPER*Flask*® Cell Culture Vessels BacMam Transduction Protocol

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Introduction

The BacMam system, commercialized by Life Technologies, uses a modified insect cell baculovirus as a reagent to transiently deliver genes into mammalian cells. This method has a high transduction efficiency within multiple mammalian cell lines resulting in high levels of protein expression and low cytoxicity. (See Kost, T.A. et al., Drug Disc. Today 2007, 12:396-403 for more information on BacMam gene expression in cells.) Here a protocol is provided for the large scale BacMam mediated transduction of HEK 293 cells using the Corning HYPER*Flask* Cell Culture Vessel and the BacMam-Enabled LanthaScreen® Histone H3 [pSer10] Cellular Assay from Life Technologies. The HYPER*Flask* Cell Culture Vessel is a multilayer flask that offers 1,720 cm^2 of growth surface (in 10 layers) while using the same spatial footprint of a T-175 flask. Two different methods, flow cytometry and Time Resolved – Fluorescent Resonance Energy Transfer (TR-FRET) are outlined, to measure transduction efficiency using the BacMam reagent encoding a GFP tagged Histone H3 fusion protein. The TR-FRET-based LanthaScreen assay technology was used to analyze specific posttranslational modifications of histones. The phosphorylation of Histone H3, specifically at serine residue 10 (Ser10), was monitored by treating cells with various dilutions of calyculin A, a serine/threonine phosphatase inhibitor, thereby stabilizing phosphorylation. The LanthaScreen assay was performed using the protocol found in the BacMam Histone H3 [pSer10] Cellular Assay User Guide from Life Technologies (Protocol, Part No. A12898pps) and phosphorylation levels were detected using the EnVision® Multilabel Plate Reader (Perkin Elmer). The method discussed in this paper can be modified for use in other cell types; it is highly recommended that a series of optimization steps be done prior to any large-scale transduction.

Helpful Hint

It is highly recommended that the protocol be read through completely prior to starting the procedure.

For handling of the HYPER*Flask* vessel, refer to the HYPER*Flask* Cell Culture Vessel Instructions for use manual can be found online at www.corning.com/lifesciences in the document library.

Cultures

Early passage Human Embryonic Kidney 293 cells (HEK 293; ATCC, Cat. No. CRL-1573™), were cultured using Iscove's Modified Dulbecco's Medium (IMDM) (Mediatech, Inc., Cat. No. 10-016-CM), supplemented with 10% Fetal Bovine Serum (FBS) (PAA Laboratories, Inc., Cat. No. A15-201) and maintained below 80 to 90% confluence. For the study, cells were scaled up using HYPER*Flask* Cell Culture Vessels (Corning, Cat. No. 10034).

Transduction

The following protocol is a modification of the Life Technologies BacMam Cellular Assay Protocol, Part No. A12898pps. All work should be done in a laminar flow hood using aseptic techniques.

1. Harvest cells using Accutase cell dissociation solution (ICT, Cat. No. 104). Transfer cell suspension into a 250 mL conical tube (Corning, Cat. No. 430776) and add equal volume of IMDM growth media to dilute dissociation solution. Centrifuge cell suspension at 270 x g for 7 minutes to remove dissociation reagent. Resuspend cells in fresh growth media to determine cell concentration and viability.
2. Prepare HEK 293 cells suspension of 9.2 x 10^4 cells/mL (30,000 cells/cm^2 in 0.326 mL/cm^2) using 10% FBS IMDM growth medium for each condition tested; mock (negative) control and HYPER*Flask*® Cell Culture Vessel (Table 1).
   a. When preparing the cell suspensions, take into account the volume of BacMam reagent that will be added to each suspension to infect the cells with the virus (Table 2).
   b. Use Corning® CellBIND® Surface treated 12 Well Cell Culture Plate (Corning, Cat. No. 3336) to run mock (negative) and internal controls.
   c. Use a 500 mL storage bottle (Corning, Cat. No. 430282) to prepare cell suspension for HYPER*Flask* vessel.
3. Using the BacMam Histone H3 [pSer10] Cellular Assay Kit (Life Technologies (Invitrogen), Cat No. A12898), add BacMam Histone H3 Reagent to HYPER*Flask* vessel cell suspension to a final concentration of 10% (v/v) (Table 2), mix well. BacMam reagent is sensitive to light; reagent addition and subsequent work should be conducted in the dark.
4. Use growth media to bring cell suspension to final seeding volume (Table 2). The HYPER*Flask* vessel cell suspension is brought up to 563 mL so that internal control samples can be removed without interfering with correct fill volume of vessel.

Table 1. Seeding Density and Media Volume for the HYPER*Flask* Cell Culture Vessel and Various Multiple Well Plate Formats

	Growth Area (cm^2)	Media Vol. (0.326 mL/cm^2)	Cell Concentration (30,000 cells/cm^2)
HYPER*Flask* Cell Culture Vessel	**1720/flask**	**560 mL/flask**	**51.6 x 10^6/flask**
24 Well Cell Culture Plate	2/well	0.650 mL/well	6.0 x 10^4/well
6 Well Cell Culture Plate	9.5/well	3.1 mL/well	2.85 x 10^5/well
12 Well Cell Culture Plate	**4/well**	**1.3 mL/well**	**1.2 x 10^5/well**
48 Well Cell Culture Plate	1/well	0.326 mL/well	3.0 x 10^4/well
96 Well Cell Culture Plate	0.32/well	0.104 mL/well	9.6 x 10^3/well

Table 2. Transduction and Mock Control Reagent Addition

	Mock (Negative) Control (3 wells)	HYPER*Flask* Cell Culture Vessel
Cell Suspension	To 9.2 x 10^4 cells/mL	To 9.2 x 10^4 cells/mL
BacMam Reagent (10%)	None	56 mL
Growth Media (Vf)	To 5.2 mL	To 563 mL

5. Mock (negative) controls; transfer 1.3 mL/well of cell suspension (without the BacMam vector) to 12 well plate, in triplicate.
6. HYPER*Flask* vessel: pour cell suspension into HYPER*Flask* vessel (refer to the HYPER*Flask* Cell Culture Vessel Instructions for use manual for instructions on how to pour liquid into the vessel).

   Internal transduction control: Transfer 1.3 mL/well of suspension from HYPER*Flask* vessel to a 12 well plate (in triplicate) as an internal control for the large scale BacMam/cell suspension mixture.
7. Incubate cultures for 48 hours in a humidified incubator set to 37°C and 5% CO_2.

LanthaScreen® Cellular Assay Setup and Transduction Verification

The LanthaScreen assay was done following the BacMam Histone H3 [pSer10] Cellular Assay kit (Cat. No. A12898) and User Guide (Protocol part no. A12898pps) from Life Technologies.

1. Verify GFP expression of transduced cells using a fluorescent microscope.
2. Prepare assay media: Opti-MEM® (Life Technologies, Cat. No. 11058) containing 0.5% dialyzed FBS (Life Technologies, Cat. No. 26400), 0.1 mM Non Essential Amino Acids (NEAA) (Life Technologies, Cat. No. 11140-050) and 1 mM Sodium Pyruvate (Life Technologies, Cat. No. 11360-070).
3. Harvest mock control cells from 12 well plate and BacMam-infected cells from the HYPER*Flask*® Cell Culture Vessel using Accutase cell dissociation solution. As mentioned above, dilute dissociation reagent using equal volumes of growth medium.
   a. Mock (negative) control cells can be harvested and pooled together to increase cell number.
   b. Internal control samples are only needed to confirm effectiveness of HYPER*Flask* vessel transduction and do not need to be harvested.
4. Remove dissociation reagent by centrifugation at 270 x g for 5 minutes. Resuspend cells in assay media.
5. Determine cell concentration and viability. If flow cytometry instrument is available, determine transduction efficiency by measuring GFP expression of cells.
6. Prepare a cell suspension at 2.5 x 10^5 cells/mL for both the HYPER*Flask* vessel and mock suspensions.
7. Transfer 20 µL/well from each cell suspension to solid white 384 well cell culture plate (Corning, Cat. No. 3570).
   a. Set up 8 well/column in triplicate (24 wells total per condition).
   b. Set up a blank (no cells) controls, by adding 20 µL/well of assay media to at least 8 wells.

   Note: Seed cells from each condition in a clear bottom 384 Well Cell Culture Plate (Corning, Cat. No. 3712) for visual imaging of cells.
8. Pulse spin plates to remove trapped air, incubate overnight in a humidified incubator set to 37°C and 5% CO_2.

LanthaScreen Cellular Assay Histone H3 Phosphorylation

1. Calyculin A (Sigma, Cat. No. C5552-10UG) was diluted in DMSO at a stock concentration of 100 mM. Prepare serial dilutions of calyculin.
2. Prepare further dilutions of the compound using assay media yielding a final DMSO concentration of 0.3%.
3. Transfer 10 µL/well from each concentration (in triplicate) to 384 assay wells (for a final DMSO concentration of 0.1%). Pulse spin plates, incubate at 37°C and 5% CO_2 for 1 hour.
4. Preparation of complete 6X Lysis Buffer:
   a. 1:100 dilution of both Protease and Phosphatase inhibitor cocktails (Sigma, Cat. No. P8340 and P0044) to 6X LanthaScreen Celluar Assay Lysis Buffer (provided with kit); mix gently.
   b. Add LanthaScreen Tb-anti-Histone H3 [pSer10] Antibody (provided with kit) at a concentration of 12 nM. Mix gently. Store in dark on ice until ready for use.
5. After 1 hour of incubation, add 6 µL/well of lysis buffer mixture to all assay wells.
6. Incubate assay plate at room temperature in the dark for 3 hours.
7. Use a fluorescent plate reader to assess the microplate. Use settings and filters for LanthaScreen assay; use Excitation 340/30 nm (PV00215), 495/10 nm (PV00315) and 520/25 nm (PV00315) along with a dichroic D400/D505 for emission.

Results of LanthaScreen® Assay

The data in Fig. 1 show a clear dose curve of different concentrations of BacMam virus used in the transduction of HEK 293 cells. Using the manufacturer's protocol for stimulating the phosphorylation of the pSer10 of the Histone protein, the data show that the use of the HYPER*Flask*® Cell Culture Vessel can be used successfully in BacMam transductions.

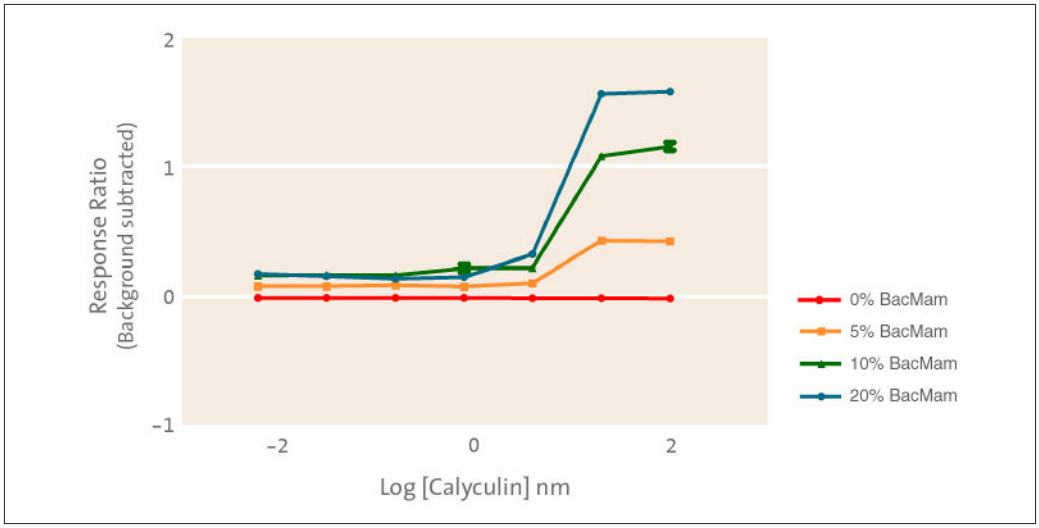

Figure 1. Optimization of BacMam virus using the BacMam-Enabled LanthaScreen Histone H3 [pSer10] Cellular Assay kit from Life Technologies.

Bibliography

BacMam Histone H3 [pSer10] Cellular Assay User Guide; Protocol, literature part number A12898pps.

BacMam Histone H3 [pSer10] Cellular Assay Product Information Sheet, literature part number A12898PIS.

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