384 Well Microplate Analysis using the BacMam Histone H3 [pSer10] Cellular Assay Kit in the Corning® HYPER*Flask*® Cell Culture Vessel

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Abstract

The high throughput analysis of potential drug candidates in a cell-based assay screen is, to say the least, complicated. New technologies are currently on the market to aid the assay biologist in developing and screening their drug candidates. Two such technologies include the BacMam Histone H3 [pSer10] Cellular Assay Kit (Life Technologies Cat. #A12898) and Corning's **H**igh **Y**ield **PER**formance Flask (HYPER*Flask*) cell culture vessel. The baculovirus-mediated gene delivery system or BacMam in combination with the LanthaScreen® assay technology can be used for the analysis of specific posttranslational modifications of histones. The HYPER*Flask* vessel is a multilayer flask that uses a gas permeable film to provide efficient gas exchange between the cells, culture medium and the environment surrounding it. The experiments presented in this poster describe a protocol combining both technologies using HEK293 cells as the model. HEK293 cells containing the GFP-Histone H3 fusion gene were seeded into Corning white 384 well normal (Corning Cat.#3570) and low volume (Corning Cat.#3826) flat bottom microplates and assayed for LanthaScreen® activity using the PerkinElmer® Envision® plate reader containing Life Technologies supplied Terbium filters. The data show successful transduction of the HEK293 cells with the GFP-Histone H3 fusion gene utilizing both technologies and resulting in a high yield production of functional GFP-Histone H3 transduced cells.

Method: BacMam Transduction

- HEK-293 cells cultured in IMDM (Mediatech Cat #10-016-CM) with 10% FBS (PAA Laboratories Cat #A15-201) were seeded at a density of 30,000 cells/cm^2 in HYPER*Flask* (HF) or HYPER*Flask*-M vessels (Cat #10024 or 10034) and 12 well Corning CellBIND® Surface multiple well plates (Cat #3336) which were used as controls.
- The protocol used in this study is a modification of the one supplied in the BacMam Histone H3 [pSer10] Cellular Assay Kit (Life Technologies Cat #12898).
- The kit supplied BacMam reagent was diluted to a concentration of 10% in IMDM with 10% FBS then a suspension of HEK-293 cells was mixed with the BacMam reagent solution as shown in Table 1. Sample volumes used for each reagent are also shown.
  - The suspension for use in the HYPER*Flask* vessel was made in a Corning 500 mL bottle (Cat #430282) while controls were made in 15 mL tubes.
- After seeding both the HYPER*Flask* vessel and 12 well multiple well plates, the samples were incubated for 48 hr in a humidified incubator set to 37ºC and 5% CO_2.

Table 1	Mock	12 well Control	HF Vessel	HF Control wells
BacMam = 10%	0	0.520 mL	56 mL	
Cell Suspension	to 9.2x10^4 cells/mL	to 9.2x10^4 cells/mL	to 9.2x10^4 cells/mL	
Media	to 5.2 mL	to 5.2 mL	to 563 mL	
Seed Volume	1.3 mL/well	1.3 mL/well	to 560 mL	1.3 mL/well of HF mixture

Mock = Non Transduced HEK-293 cells
12 well Control = Small scale transduction in 12 well multiple well plates
HF (HYPER*Flask*) Vessel Control = Cells from transduction performed in HYPER*Flask* vessel and then plated in 12 well multiple well plates

HEK-293 Transduction Efficiency and Viability

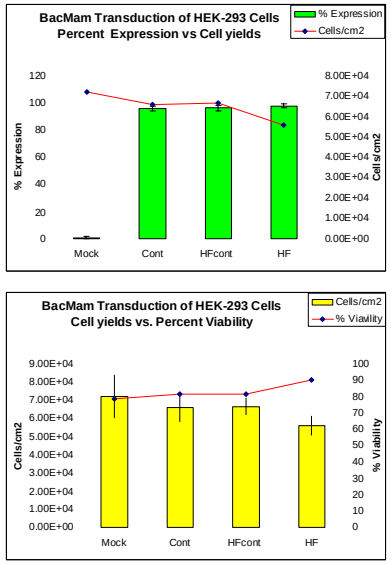

Figure 1: Confirmation of transduction efficiency (A) and HEK-293 viability (B). Percent Green Fluorescent Protein (GFP) expression was determined using the Guava Minicyte Flowcytometer (Millipore). The data presented is an average of 4 independent studies. Cell yields (cells/cm^2) determined by trypan blue exclusion using a Vi-CELL® XR Cell Viability Analyzer (BeckmanCoulter). Greater then 95% of transduced cells showed GFP expression as determined by Flow Cytometry (**A**) with > 80% viability in the control and HYPER*Flask* vessel samples (**B**). No difference in transduction efficiency between HYPER*Flask* vessel samples and controls was observed.

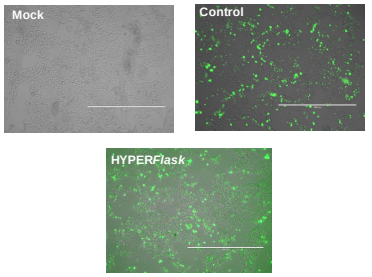

Figure 2: Green Fluorescent Protein (GFP) expression was verified microscopically (4x magnification) using an EVOS®$_{fl}$ microscope (Advanced Microscopy Group).

Method: LanthaScreen® Assay

To further asses the effectiveness of the BacMam transduced HEK-293 cells, post-translational modification of histones was confirmed using the LanthaScreen® technology. The ease of assay miniaturization was also demonstrated using Corning's normal volume (Cat #3570) and low volume (Cat #3826) 384 well microplates.

The transduced HEK-293 cells were harvested then resuspended in fresh assay media. The assay medium is composed of Opti-MEM® medium (Life Technologies Cat #11058) containing 0.5% FBS (Life Technologies Cat #12676-011), 0.1 mM NEAA (Mediatech Cat #25-025-CI) and 1 mM Na Pyruvate (Mediatech Cat #25-000-CI). The LanthaScreen® cellular assay was set up following the Life Technologies protocols (Literature part #A12898PIS and A12898PPS) with the following modification:

- The Calyculin A (Sigma Cat #C5552-10UG) was diluted in DMSO at a starting concentration of 100µM and subsequent dilutions made in Assay medium. The final DMSO concentration used in the assay was of 0.3%.
- Add 10µL/well of each Calyculin A dose (in triplicate) to 384 normal volume plates.
- For the low volume microplates 10µL was removed of media and replaced with 5 µL/well of each Calyculin A dose as stated above.
- Pulse spin plates, incubate in a humidified incubator set to 37C and 5% CO_2 for 1hour.
- Add the 6µL of 6X Lysis buffer to the normal volume and 3 µL to the low volume microplate. Pulse spun plates then incubate at room temperature in the dark for 3 hours.

The signal from the microplate wells was read using a PerkinElmer EnVision® Multilabel Reader selecting the LanthaScreen® filters. The filters used for the terbium and GFP paired TR-FRET assays were, for excitation 340/30 nm (PV00215), emission 495/10 nm (PV00315), and 520/25 (PV00315) along with a dichroic D400/D505.

Histone H3 Phosphorylation Using the LanthaScreen® System

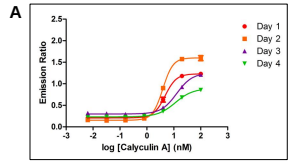

Table 2	Day 1	Day 2	Day 3	Day 4
Control	4.733	3.881	12.83	11.32
HYPER*Flask* vessel	8.423	6.658	11.89	12.62

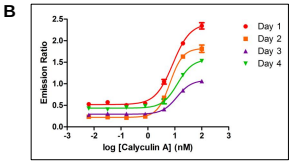

Figure 3. Histone H3 Phosphorylation LanthaScreen® Assay. The data represents the LanthaScreen® assay results of the BacMam transduction comparing Mock, HYPER*Flask* control and HYPER*Flask* samples. The Graphs represent the Emission ratio of the Control samples (A) and HYPER*Flask* (B) plated as four independent experiments to show the relative consistency between assays. Table 2 shows the EC_{50} for each independent experiment.

384 Well Microplate Normal versus Low Volume Comparison

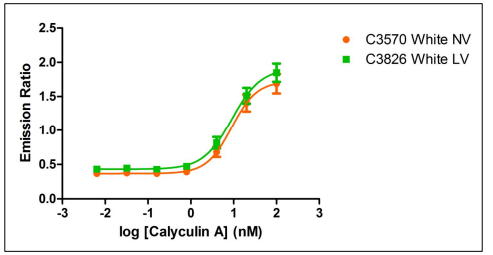

Table 3	Max-Min Signal	Signal:Noise	EC50
C3570 White NV	1.32	4.6	9.03
C3826 White LV	1.41	4.26	9.16

Figure 4. Histone H3 Phosphorylation LanthaScreen® Assay 384 Normal versus Low Volume Comparison. The data show that there is no difference comparing maximal signal detected, signal:noise or EC_{50} between the microplate formats.

Summary/Conclusions

- The protocol for transducing HEK-293 cells using the BacMam Histone H3 [pSer10] Cellular Assay Kit with Corning's High Yield PERformance Flask (HYPER*Flask*) cell culture vessel is shown.
- The high efficiency of the protocol for use with the HYPER*Flask* vessel, as measured by GFP expression, results in a cell line where post-translational modification of Histones can be measured. Performing the transductions in the HYPER*Flask* vessel will allow for screens where high cell density is required.
- Cells transduced with the BacMam virus in the HYPER*Flask* vessel can be used in the LanthaScreen® assay with no statistical impact compared to those cells transduced and plated in 12 well multiple well plates.
- The LanthaScreen® assay can be run in both normal and low volume 384 well white microplates with no change in signal quality. The ability to miniaturize the assay will allow for greater savings in cost and reagents.