MultiFlow®

DNA Damage Kit – p53, γH2AX, Phospho-Histone H3

App Note 1:
Assay Installation

Start out the right way

Introduction

The MultiFlow assay provides a significant step forward in the assessment of genotoxicity with multiplexed, high throughput information. This DNA Damage Kit provides you with the reagents and instructions to process and analyze cells using your own flow cytometer. Results include a measure of both genotoxicity and Mode of Action (clastogen or aneugen).

You're in Good Hands

Setting up this assay in your lab doesn't have to be something you do alone. How do you ensure reliable and reproducible data across different labs, different instruments, and over time?

The solution is to perform our standard "Assay Installation". This App Note will describe the general aspects of a MultiFlow Installation and is applicable to many different flow cytometers.

On Site or Remote Installation

Assay installation can be accomplished either remotely or as part of an on-site visit. Scheduling on-site installation typically gets you up and going faster, while giving you the opportunity for hands-on training in other aspects of the assay. Remote installation may require more time to complete, but can be a better option for some groups.

Voltage Set-Up

The first step of installation is to calibrate the instrument using the Nuclei Release Solution provided in the MultiFlow kit. Adjust the photomultiplier tube (PMT) voltages for each channel so that the fluorescent beads achieve specific target values. These values are determined by your specific flow cytometer's settings. Once these adjustments have been made, save these instrument settings for future use.
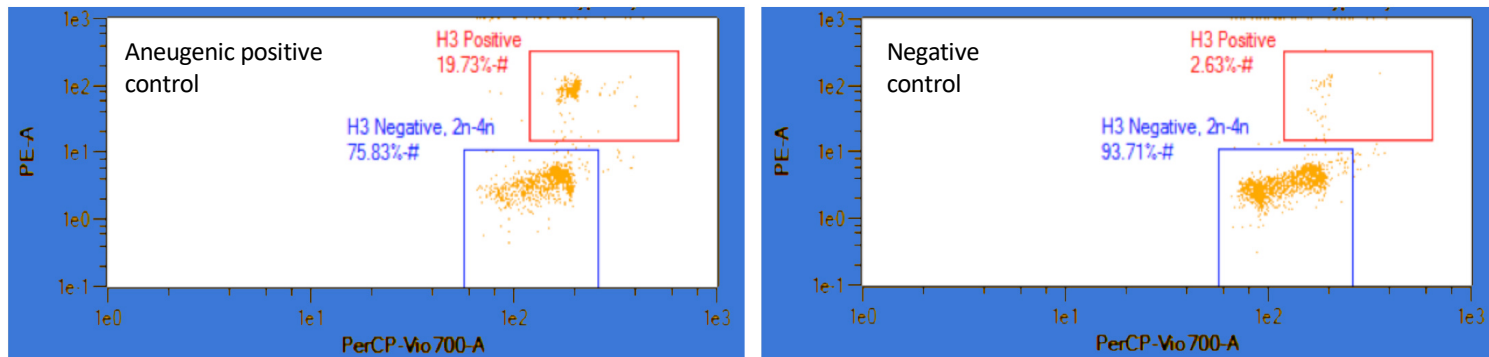
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Figure 1A (left), 1B (right): Positive and negative control samples assist in identifying populations and appropriately setting gates.

Compensation Set-Up

The next step is necessary to eliminate spill-over from overlapping fluorescence profiles. Use MultiFlow reagents to process both a negative control sample and an aneugenic positive control sample. Once these samples are ready to be analyzed, you can begin adjusting compensation.

While running the aneugen positive control sample, adjust the compensation controls for the PE and PerCp channels to bring the populations into the desired positions (Figure 1A). The compensation settings are then confirmed by running the negative control sample (Figure 1B). Examples of over- and under-compensation are shown in Figures 2A and 2B. It's important to ensure adequate separation of the positive and negative events.

Once these adjustments have been made, save these instrument settings for future use. Additional fine-tuning of gate positions should be performed at this point. For remote installations, example plots should be sent to Litron to confirm optimal settings.
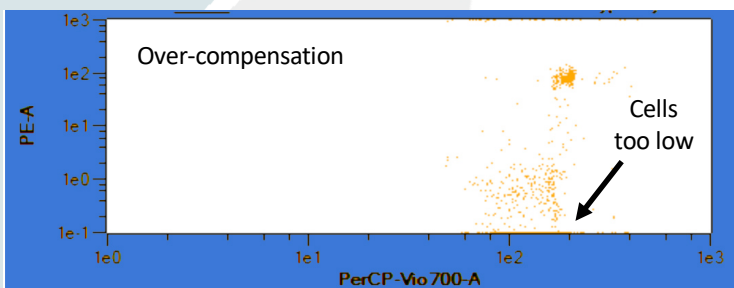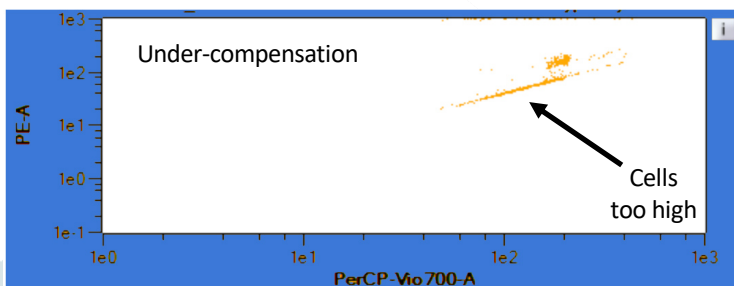

Figure 2A (top), 2B (bottom): Adjusting compensation may require analysis of more than one sample.

Conclusions

At this point, the installation process is complete. It should not need to be repeated unless significant service is performed on your flow cytometer.

Next app note in the series:
- Build training and test sets of MultiFlow data by analyzing prototypical compounds
- Create lab-specific algorithms for the prediction of genotoxic mode of action

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