A Flexible Solution for Automating Cell Staining Using the Biomek 4000 Workstation

Amy Yoder¹, Li Liu¹, William Godfrey²
Beckman Coulter, Inc., ¹Indianapolis, IN, ²Miami, FL

Abstract

The automated Cell Staining Application on the Biomek 4000 Workstation can improve throughput and reproducibility of conventional staining of live and fixed adherent cells. This application provides a walk-away solution for staining cells in a variety of workflows, including antibody staining, nuclear dyes, and viability assays in a 96-well plate. Flexibility is central to this application, allowing users to choose and customize any combination of steps (fixation, permeabilization, washing, blocking, and staining) by configuring options in a simple User Interface. Manual staining of cells, either to characterize antigen expression or to assess cell health, is labor-intensive and time-consuming. Here we demonstrate several automation strategies using the Cell Staining Application on the Biomek 4000 Workstation.

Cell staining applicability is demonstrated with three models using mouse embryonic stem cells (mESCs). Mouse embryonic stem cells (mESCs) were stained with either anti-Nanog-Alexa Fluor 488 (clone eBioMLC-51, eBioScience) or anti-Sox2-Alexa Fluor 647 (BD Biosciences, San Jose, CA) yield CV’s of 6.6% and 7.6%, respectively. The staining was performed on the Biomek 4000 Workstation using the Cell Staining Application. The percentages of positively stained cells (Table 1) were quantified using the MetaXpress software. The standard deviation and coefficient of variation are based on 24 replicate samples.

Materials and Methods

Marine Embryonic Stem Cell (mESC) Growth and Differentiation

Mouse embryonic stem cells (mESCs) were cultured in growth media containing leukemia inhibitory factor (LIF) and 15% knockout serum replacement (KSR). For differentiation, cells were cultured in 15% FBS without LIF in a 384-well round bottom polystyrene plate (Nunc, Roskilde, Denmark) in 40 µL differentiation medium (various treatments for 0 to 7 days). Embryoid bodies formed by Day 5 were transferred to gelatin-coated 96-well plates in 100 µL fresh media. After Day 6, a portion of the adherent cells showed visible contraction. Control mES cells were treated in parallel without differentiation factors. Cells were harvested by Trypsin and dispersed into fresh 96-well plates, allowed to adhere for 24 hours prior to staining.

Cellular Staining

Cytometric analysis was performed on a flow cytometer (BD FACSCalibur; BD Biosciences), and data analysis was done using FlowJo software. Results were analyzed on an ImagXpress system with MetaXpress software.

Apoptosis Characterization

Staining for caspase-3 and -7 was performed using an antibody cocktail (clone 788T, BD Biosciences). The results clearly demonstrate the expected increase in apoptotic cells as staurosporine is increased (Figure 6). The mixed cells were fixed and permeabilized with a mixture of mES cells and feeder cells, then allowed 24 hours to plate out before being treated with PerFix-nc reagents and stained using the Biomek 4000 Workstation. Sixteen wells required less than an hour to prepare for imaging. Images were taken using the 10X objective on an ImagXpress high content imager.

Results

mES Cell Staining

The mixture of mES cells and feeder cells were stained with either anti-Nanog-Alexa Fluor 488 (clone eBioMLC-51, eBioScience) or anti-Sox2-Alexa Fluor 647 (clone O30-678, BD Biosciences) (Figure 5). All conjugates were labeled for optimal performance, and relevant isotype controls were used to control for non-specific staining. Staining was done manually and on the Biomek 4000 Workstation using the Cell Staining Application. The percentages of positively stained cells (Table 1) were quantified using the MetaXpress software. The standard deviation and coefficient of variation are based on 24 replicate samples.

Cardiomyocyte Staining

Differentiated cells were harvested on Day 8 using Accutase (Milipore, Billerica, MA). Then mES cells were fixed, and permeabilized with PerFix-nc reagents. The mixture of differentiated cells and mES cells were stained with anti-crem Heavy chain-Alexa Fluor 488 (clone MF20, eBioScience) and anti-Sox2-Alexa Fluor 647 (Figure 7). All liquid handling steps were performed on a Biomek 4000 Workstation. Sixteen wells required less than an hour to prepare for imaging. Images were taken using the 10X objective on an ImagXpress high content imager.

Annexin V FITC/4′,6-Diamidino-2-phenylindole (DAPI)

The staurosporine treated cells were stained with Annexin V/FITC/7-ADD and DAPI on the Biomek 4000 Workstation using the Cell Staining Application (Figure 7). Ten replicates of each staurosporine concentration (0, 0.5, 1, and 2 µM) were analyzed for a total of 40 samples (Figure 8). The automated staining process for these 40 samples required approximately 1.5 hours with no user intervention.

Conclusion

Cell staining workflows can be automated using standard components on the Biomek 4000 Workstation. The flexible application can be customized for a variety of staining workflows. Automation can achieve preparation time savings with large numbers of samples while maintaining equivalent results and precision compared with manual processing. It is possible to perform large cell staining studies in an expandable manner with walk-away capability.