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

Materials and Methods

Marine Embryonic Stem Cell (mESG) Growth and Differentiation

Mouse embryonic stem (mES) cells (Life Technologies) were maintained 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 5 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 dispensed into fresh 96-well plates, allowed to adhere for 24 hours prior to staining.

Cellular Staining

Cells were fixed by adding 5 μL of PerFix-nc reagent (B10825, Beckman Coulter, Brea, CA) for 15 minutes, followed by permeabilization and staining with 50 μL of PerFix-nc reagent 2 containing the antibody conjugates for 30 minutes. After the removal of the supernatant and replacement with 50 μL reagent 3 for 5 minutes, cells were identified by nuclear staining with mounting medium which contains DAPI (Life Technologies).

Apoptosis Characterization

Stem cells were seeded into 96-well imaging plates as above, and then treated with doses of staurosporine (Life Technologies) for 7 hours. The treated cell cultures were stained with the annexin V-FITC / 7-AAD (M3614, Beckman Coulter). Cells were identified by nuclear staining with Hoechst 33342 (Life Technologies).

Imaging

All samples were analyzed on an ImagiXpress system with MetaXpress software (Molecular Devices, Sunnyvale, CA).

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 6). All conjugates were titrated 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 Accumax (Milipore, Billerica, MA). Then mES cells were mixed with either pooled differentiated cells or feeder cells (Life Technologies), and seeded into 96-well imaging plates and allowed 24 hours to attach. The mixed cells were fixed and permeabilized with PerFix-nc reagents. The mixture of differentiated cells and mES cells were stained with myosin 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 ImagiXpress high content imager.

Figure 7: Cells were stained with either isotype controls (a.) or with myosin heavy chain (green, b.) and Sox2 (blue, c.). DAPI (red, d.) was used to mark the locations of nuclei.

Annexin V / 7-AAD Staining

The staurosporine treated cells were stained with Annexin V-FITC/7-AAD on the Biomek 4000 Workstation using the Cell Staining Application (Figure 8). Ten replicates of each staurosporine concentration (0 μM, 0.5 μM, 1 μM and 2 μM) were analyzed for a total of 40 samples (Figure 9). The automated staining process for these 40 samples required approximately 1.5 hours with no user intervention.

Figure 8: (right) Example Images of Annexin V–FITC/7-AAD staining on staurosporine treated mES cells. (left) Staurosporine (0, 0.5, 1 & 2 μM) treated mES cells images: transmitted light images, and composite images with Annexin V–FITC (green), 7-AAD (red) and Hoechst 33342 (blue).

Figure 9: Fold increase in apoptosis with increase in staurosporine concentration. n=10 for each concentration.

Conclusion

- Cell staining workflows can be automated using standard components on the Biomek 4000 Workstation.
- The flexible automation 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.