

Immunostaining Cells in the M1 Microfluidic Plate

The ability of our system to automate rapid flow switching in a cell culture environment lends itself as a useful alternative to traditional labor-intensive procedures for immunostaining cells. The below protocol offers an example of how the ONIX system can be used to immunostain α -tubulin, as well as the nucleus and actin filaments, of different cell types easily and efficiently.

Protocol

1. In a new M1 plate, load cells via surface tension loading through the cell loading well E2. (See the “M2 Microfluidic Plate” document for more detailed instructions on surface tension cell loading). Culture under gravity flow for at least 24 hours, giving the cells time to equilibrate with their environment.
2. Take the prepared M1 plate and aspirate all wells: A1, A2, B1, B2, C1, C2, E1 and E2. Add 100-200 μ l of each solution to the wells as specified in Table 1. Seal this plate to the ONIX manifold and open the ONIX software.
3. Import the file “M1 Immunostaining.txt” and click “Test.” Verify that the program will take 3 hours and 30 minutes by clicking “OK.” Also check to ensure that all solutions are set to be driven by a pressure of 3 PSI. Click “Start” to run the program.
4. After the program has finished, unseal the plate and aspirate all wells. *Optional: For longer-lasting fluorescence, add 2-3 drops of ProLong® Gold antifade reagent (Invitrogen) to the E2 well, reseal the plate, and pressurize E2 at 3 PSI for about 1 hour. Unseal when finished.*
5. View cells with a standard fluorescence microscope using the DAPI, FITC and CY3 filters to image the cells’ nuclei, microtubules and actin filaments, respectively, in the M1 chamber. See Figure 1 for images taken of immunostained MCF-7, PC-3 and NIH 3T3 cells.

Figure 1: Fluorescent microscope images (40x) taken of cells immunostained according to this protocol **a.** MCF-7 cells, **b.** PC-3 cells, and **c.** NIH 3T3 fibroblasts.

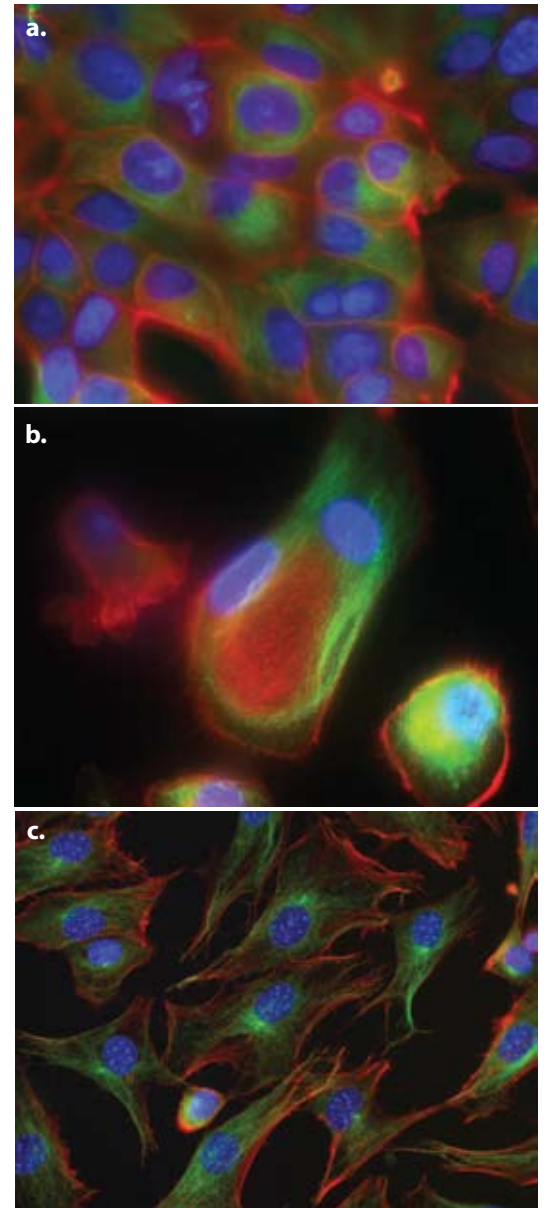


Table 1: Add 100-200 μ l of the specified solutions to the respective wells before running the ONIX program. It is best to prepare the primary and secondary antibody solutions fresh before each use to ensure robust fluorescence.

Well	Solution	Purpose	Details
A1	PBS	Washing	Phosphate buffered saline
A2	Paraformaldehyde	Fixing cells	4% Formaldehyde
B1	PBT	Permeabilize cells	0.01% TritonX-100 in PBS
B2	10% NGS	Block cells	10% non-immune normal goat serum (Invitrogen)
C1	Primary Antibody Solution	Bind α -tubulin, Stain nuclei	1 μ g/mL anti- α -tubulin (bovine) mouse IgG, 10 μ g/mL Hoechst 33342, in 10% NGS (Invitrogen)
C2	Secondary Antibody Solution	Tag α -tubulin, Stain actin filaments	1% BSA, 5 μ g/mL Alexa Fluor® 488 goat anti-mouse IgG (H+L), 8 mg/mL Alexa Fluor 546 phalloidin, in PBS (Invitrogen)