FLOW CYTOMETRY PROTOCOL FOR NUCLEAR ENVELOPE AND NUCLEAR MATRIX TARGETS

The following flow cytometry protocol for staining intracellular nuclear targets using detergents to permeabilize cell membranes has been developed and optimized by Bio-Techne. Individual experimental designs for flow cytometry must be optimized, including antibody dilution and incubation time but it is recommended to use 1 x 10^6 cells per 100 µL of sample. For low cell density or poorly expressed intracellular targets, techniques like Single-Cell Westerns may be advantageous. Learn more about Milo™ here. *Please read the protocol in its entirety before starting.*

Materials

- 1 X PBS (0.137 M NaCl, 0.05 M NaH^2PO^4, pH 7.4)
- Flow Cytometry Fixation Buffer (R&D Systems, Cat No. FC-004) or an equivalent solution containing 1 - 4% paraformaldehyde
- Permeabilization Buffer: 0.1–1% Triton or NP-40 in PBS
- Flow Cytometry Staining Buffer (R&D Systems, Cat No. FC-001) or an equivalent solution containing BSA and sodium azide
- Fc Receptor Blocking Reagents (These include Fc receptor blocking antibodies or IgG solutions)
- Primary Antibodies
- Isotype Control Antibodies
- **Optional:** Secondary Antibodies
- **Recommended viability dye:** DAPI, Novus Biologicals, Cat No. NBP2-31156; Propidium Iodide, Novus Biologicals, Cat No. NBP2-31155; or 7-AAD, Novus Biologicals, Cat No. NBP2-294 46
- Trypan Blue
- FACS Tubes (5 mL round-bottom polystyrene tubes)
- Pipettes with appropriate tips
- Centrifuge
- Vortex

Sample Preparation

Sample Type	Suggestions
Cells in Suspension	After removing media from suspended cells, add cold PBS to remove residual growth factors from cell culture media. After washing media remnants, use cells suspended in PBS and proceed with washing in Step 2.
Adherent Cells	After removing media from adherent cells, add cold PBS to remove residual growth factors from cell culture media. • Harvest cells with a 1% BSA solution in PBS and then proceed with washing in Step 2. • Adherent cell lines may require 0.5 mM EDTA to facilitate removal and then washed according to Step 2. *Exposure time with EDTA should be minimal.*
Tissue	To prepare tissues for flow cytometry, mechanical and/or enzymatic disaggregation is required. • First, mince the tissue into small sections that expose the cells and suspend in PBS. *Enzymatic digestion may be required after mincing the tissue, but digestion buffer will be tissue type dependent.* • Next, pass the minced tissue suspension through a fine gauge needle several times until all cells are fully in suspension. *If you experience resistance, exchange needle with a larger gauge to dissociate cells first.*

NOVUS BIOLOGICALS
a bio-techne brand

FLOW CYTOMETRY PROTOCOL FOR NUCLEAR ENVELOPE AND NUCLEAR MATRIX TARGETS

Methods

1. Harvest your cells (see Sample Preparation for guidance).
2. Add 2 mL of PBS with a pipette to wash cells. Centrifuge at 1,300 RPM (500 x g maximum) and 4°C for 5 minutes, decanting the supernatant. Wash 3 times.
3. Using a small aliquot, count the cells. Using a hemocytometer and a 1:1 Trypan Blue exclusion stain, count cells to determine cell viability before starting.

   TIP: Surface staining should be performed now. Cell viability staining should be done prior to cell fixation. If only nuclear staining is performed, proceed with cell fixation.

4. Add 500 µL of cold Fixation Buffer into FACS tubes required for your experiment. Aliquot up to 1 x 10^6 cells/100 µL. *A separate set of cells should be stained with an isotype control antibody as a negative control.*
5. After adding the cells to 500 µL of cold Fixation Buffer per sample tube, vortex to mix. Incubate the cells at room temperature (RT) for 10 minutes.
6. Centrifuge cells at 1,300 RPM and 4°C. Decant the Fixation Buffer.
7. Resuspend each cell pellet in 150 µL Permeabilization buffer (0.1–1% Triton or NP-40 in PBS). Add 1 µg blocking IgG per 1 x 10^6 cells and let stand for 15 minutes at RT. *Do NOT wash excess blocking IgG from this reaction. It is important to keep cells in Permeabilization Buffer during intracellular staining.*
8. Add 5-10 µL of conjugated antibody (or a previously determined amount) per 1 x 10^6 cells and vortex. *For unconjugated antibodies, be sure to check the data sheet for any appropriate concentrations validated for use in flow cytometry. 1 µL of primary antibody per 1 x 10^6 cells is a good starting point.* Incubate cells for 30 minutes at 4°C protected from light.
9. Add 2 mL of Permeabilization Buffer to wash cells ONCE. Centrifuge at 1,300 RPM (500 x g maximum) and 4°C for 5 minutes, decanting the supernatant.

   TIP: If an unconjugated primary antibody was used, incubation with an appropriate secondary antibody is required. After washing cells to remove the primary antibody, resuspend the cell pellet in 150 µL of Flow Cytometry Permeabilization/Wash Buffer I. Add the recommended volume of secondary antibody and incubate for 30 minutes protected from light. Gently vortex intermittently to maintain a single cell suspension. Wash cells ONCE using Flow Cytometry Permeabilization/Wash Buffer I instead of PBS.

10. Resuspend the cell pellet in 200 – 400 µL of Flow Cytometry Staining Buffer for flow cytometry analysis.