# Primary and Secondary Labeling of cells on USF Nanofilm Plates

**Supplies needed:**

- Cells of Interest
- USF Nanofilm Plate
- PBS (1X, pH7.4)
- Saponin
- Normal Goat Serum (5% in PBS)

**Protocol:**

This protocol has been optimized with cells plated 48hrs prior to fixation.

- See [Cell Plating Protocol for USF Nanofilm Plates] for guidance on plating cells on USF plates.
- See [Paraformaldehyde fixation of cells on USF Nanofilm Plates] for guidance on cell fixation.

Blocking:

1. Prepare blocking solution:
   - Blocking solution = 5% Normal goat serum (NGS) in PBS with 0.1% saponin solution.
2. Aspirate solution on cells carefully without touching or scraping nanofilm surface.
   - Note: Perform wash steps quickly. Nanofibers should not be allowed to dry out; drying may cause fiber clumping and alter scaffold behavior.
3. Add 300 – 500 uL of 5%NGS + 0.1% saponin solution to each well.
   - Note: Avoid disturbing the nanofilm surface during pipetting; dispense media against the side of the well when possible.
4. Incubate at 25°C for 30 minutes.

Primary Antibody Labeling:

5. Prepare primary antibody solution:
   - Primary antibody solution = 5%NGS + 0.1% saponin solution + Primary antibody
   - Typical primary concentration ~ 1:200 to 1:500 dilution.
6. Remove blocking solution and replace with 350uL of primary antibody solution.
7. Incubate O/N at 4°C.

Secondary Antibody Labeling:

8. Prepare secondary antibody solution:
   - Secondary antibody solution = 5%NGS + 0.1% saponin solution + Secondary antibody
   - Typical primary concentration ~ 1:500 dilution.
9. Aspirate primary antibody solution carefully and add 0.5 mL PBS.
10. Repeat wash two additional times (total of three washes).
11. Add 350uL secondary antibody solution to well and incubate at 25°C for 2-4 hours.
12. Aspirate antibody solution carefully and add 0.5 mL PBS.
13. Repeat PBS wash two additional times (total of three washes).
14. Add 0.5mls PBS and proceed to downstream workflow store for later use.
    - Storage: Seal the plate with Parafilm to minimize evaporation and store at 4 °C, protected from light. Keep wells filled with PBS.

**Additional Notes:**

- Keep nanofilms wet at all times. Drying may cause fiber clumping and alter scaffold behavior.
- It is recommended to wait at least 48 hours after plating cells to achieve optimal imaging conditions.
- Concentrations and incubation times may need to be adjusted depending on cell type and antibodies.
- Nanofilms are also compatible with Triton-X permeabilization.