

Extracellular Vesicle (EV) Staining on a Blank Chip

A. Surface Preparation and EV Capture

1. Poly-L-lysine (PLL) Coating: Add 0.1% PLL to the blank chip surface and incubate for 20 minutes at room temperature (RT).
2. Rinse: Wash gently with 100 μ L distilled water to remove excess PLL.
3. EV Loading: Add EV suspension (1×10^8 to 1×10^{11} particles) and incubate for 1 hour 15 minutes at RT.
4. Wash Step: Wash with 1 $\times$ PBS to remove unbound EVs.

B. Antibody Staining Procedure

5. Initial Fixation: Fix with 4% PFA for 10 minutes at RT.
6. Wash: Rinse with 100 μ L PBS.
7. Blocking: Incubate with 5% BSA blocking/staining solution for 15 minutes (keep solution at 4°C before use).
8. Primary Antibody Incubation: Add antibodies diluted in staining solution (final 1–10 μ g/mL) and incubate for 50 minutes at RT.
9. Wash: Wash with PBS.

C. Post-staining Fixation and Storage

10. Secondary Fixation: Fix with 4% PFA for 5 minutes.
11. Final Wash: Wash with PBS.
12. Imaging or Storage: Image immediately or store in PBS at 4°C overnight.

Notes

- Perform all washes gently to avoid detaching EVs.
- Keep samples hydrated; do not allow the chip to dry.
- Use low-retention tips when handling EVs.
- Protect fluorescent antibodies from light.