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 µL 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× 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 µL 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 µg/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.