

Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

**High-throughput fluorescence correlation spectroscopy enables analysis
of surface components of cell-derived vesicles**

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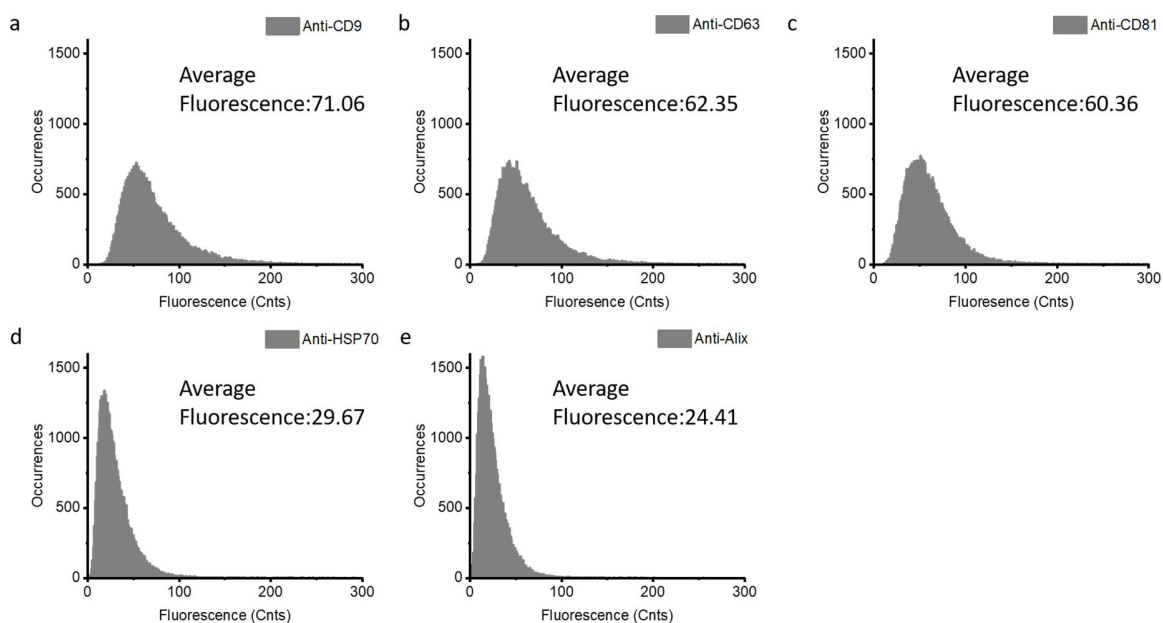


Fig. S1 Calculated mean fluorescence intensity of free antibodies from anti-CD9-CF543 antibody (a), anti-CD63-CF543 antibody (b), anti-CD81-CF543 antibody (c), anti-HSP70-CF543 antibody (d), and anti-Alix-CF543 antibody (e)

Table S1 The comparison of different methods used in the studies of vesicle characterization

FEATURE	HT-FCS	FLOW CYTOMETRY	WESTERN BLOT	MASS SPECTROSCOPY
WHAT IS MEASURED	Fluorescence fluctuations	Enhanced forward scatter	Immunoblotting	Mass-to-charge ratio of ions
PARTICLE SIZE	Yes	Yes	No	No
CONCENTRATION	Yes	Absolute number	Relative concentration	No
PROTEIN COMPOSITION	Yes (with surface marker)	Yes (with surface marker)	Yes	Yes
SINGLE PARTICLE DETECTION	Yes	Yes	No	No

Table S2 Antibody binding analysis among 4 different cell-derived vesicles

	A549				HEK293T						N2A				Raw 264.7					
Diffusion Coefficient ($\mu\text{m}^2/\text{s}$)	1.2	1.2	8.9	16	1.9	0.8	1.6	1.1	14	2	14	16	16	10	6.1	12	18	15	10	0.8
Average bound antibodies	2.1	1.9	n/a	n/a	2.1	2.5	1.8	1.3	n/a	2.6	n/a	n/a	n/a	n/a	1.6	n/a	n/a	n/a	n/a	3.9
Diffusion Time (ms)	23	23	3	1.7	14	34	17	24	1.9	15	1.9	1.7	1.7	2.2	4.6	2.3	1.5	1.8	2.2	34
Binding	✓	✓			✓	✓	✓	✓		✓					✓					✓