Supplementary Figure S1

Fig 1 raw data of Particle tracking analysis (PTA) using ViewSizer 3000

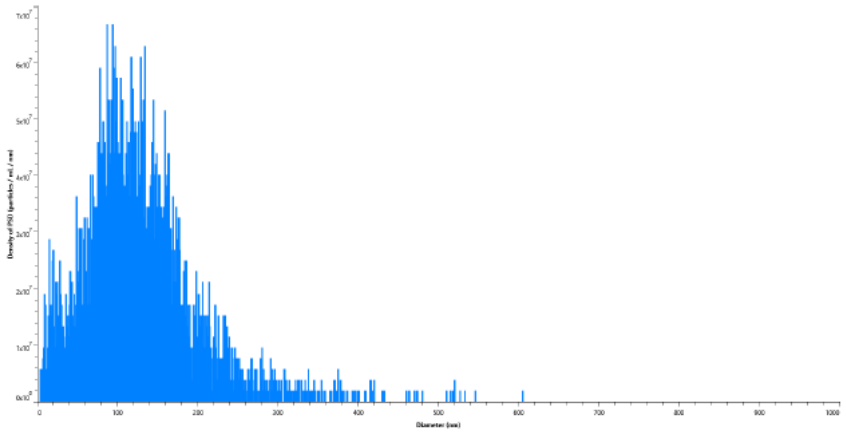
A. Particle concentration and particle size of VLSV prepared by new protocol

HORIBA Scientific

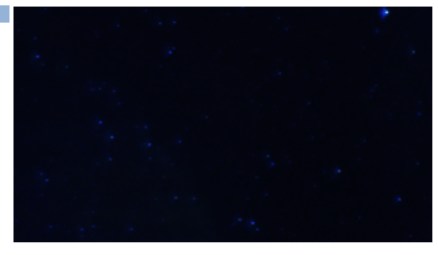
ViewSizer 3000 - Nanoparticle Size Analyzer

Sample information					
Sample name	takayama_method_x100	**Analyst**		**Recording date**	2026-02-03 15:09:02
Method	Exosomes	**Sample type**	Sample		
Sample dilution	100	**Sample amount**	1 mg	**Density**	2 g/cm³
Blank name	N/A	**Blank concentration**	0.00×10³ particles/mL		
File path	D:\HORIBA\VU-3000\Data\20260203\takayama_method_x100\takayama_method_x100_20260203_151517.xml				
Blank file path	N/A				
Description	None				

Acquisition information	Multispectral Particle Tracking Analysis (MPTA)						
Blue laser (445 nm)	70 mW	**Stirring time**	3 s	**Focus**	10.293 mm	**Dispersant**	PBS
Green laser (520 nm)	12 mW	**Stirring speed**	1400 rpm	**Gain**	24 dB	**Viscosity**	0.9744 (mPa·s)
Red laser (635 nm)	8 mW	**Wait time**	2 s	**Exposure**	24 ms	**Refractive index**	1.34
Pulse duration (Blue)	24 ms	**Temperature**	22 °C	**Frame rate**	30 fps		
Pulse duration (Green)	24 ms	**Filter type**	None	**Frames/video**	300		
Pulse duration (Red)	24 ms	**Wavelength**	N/A	**Videos count**	25		



Parameter	Result
Mean	139.56 nm
Mode	87.44 nm
D10	72.83 nm
D50	126.77 nm
D90	220.67 nm
Span	1.166 nm
Concentration (sample)	6.08×10⁸ particles/mL
Concentration (minus blank)	6.08×10⁸ particles/mL
Integration range	50 - 500 nm
Chart type	ConstantBinsTable
Volume calibration	None



Video processing information					
Detection threshold	2	**Drift correction**	0%	**Std. dev./CoV**	65 nm / 46.6%
Detection type	Polydisperse	**Calibration constant**	197.01 ± 0.1 (nm/pixel)	**Camera type**	AVT Manta 319C
Cluster algorithm	Overridden	**Processing date**	2026-02-03 15:15:19	**Volume**	2.10×10⁻⁶ (mL)
Feature radius	40 pixels	**Total/proc. frames**	7500 / 7500	**Software/firmware**	2.61 / 1.3.5
Tracking starts on	1 frame	**Total/in range counts**	3570 / 3190	**Serial number**	0052

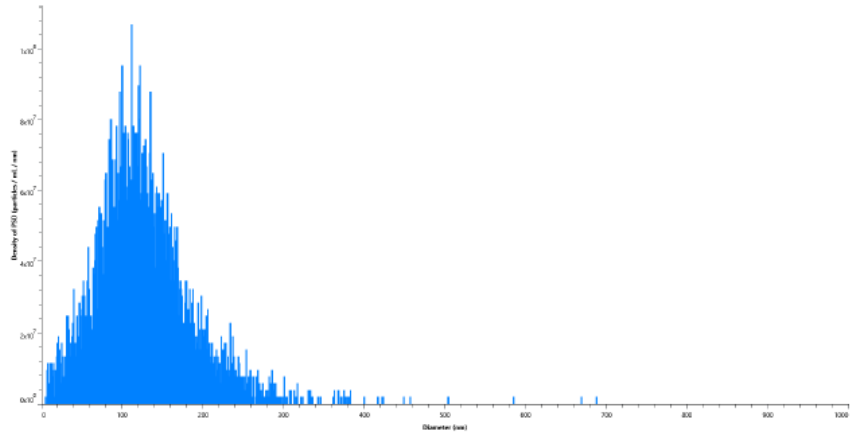
B. Particle concentration and particle size of VLSV prepared by old protocol

HORIBA Scientific

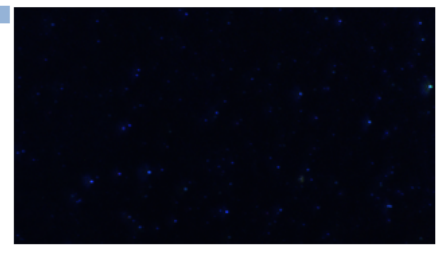
ViewSizer 3000 - Nanoparticle Size Analyzer

Sample information					
Sample name	sakai_method	**Analyst**		**Recording date**	2026-02-03 15:40:59
Method	Exosomes	**Sample type**	Sample		
Sample dilution	100	**Sample amount**	1 mg	**Density**	2 g/cm³
Blank name	N/A	**Blank concentration**	0.00×10³ particles/mL		
File path	D:\HORIBA\VU-3000\Data\20260203\sakai_method\sakai_method_20260203_154716.xml				
Blank file path	N/A				
Description	None				

Acquisition information	Multispectral Particle Tracking Analysis (MPTA)						
Blue laser (445 nm)	70 mW	**Stirring time**	3 s	**Focus**	10.278 mm	**Dispersant**	PBS
Green laser (520 nm)	12 mW	**Stirring speed**	1400 rpm	**Gain**	24 dB	**Viscosity**	0.9744 (mPa·s)
Red laser (635 nm)	8 mW	**Wait time**	2 s	**Exposure**	24 ms	**Refractive index**	1.34
Pulse duration (Blue)	24 ms	**Temperature**	22 °C	**Frame rate**	30 fps		
Pulse duration (Green)	24 ms	**Filter type**	None	**Frames/video**	300		
Pulse duration (Red)	24 ms	**Wavelength**	N/A	**Videos count**	25		



Parameter	Result
Mean	134.66 nm
Mode	112.50 nm
D10	74.54 nm
D50	125.08 nm
D90	206.20 nm
Span	1.053 nm
Concentration (sample)	8.29×10⁹ particles/mL
Concentration (minus blank)	8.29×10⁹ particles/mL
Integration range	50 - 500 nm
Chart type	ConstantBinsTable
Volume calibration	None

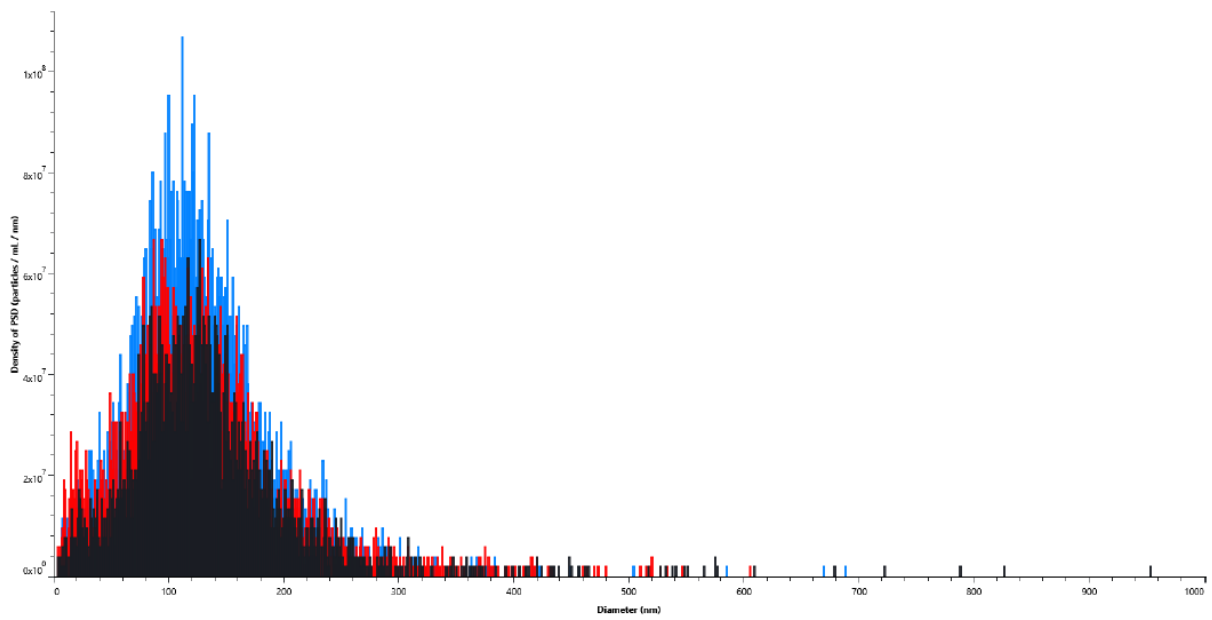


Video processing information					
Detection threshold	2	**Drift correction**	0%	**Std. dev./CoV**	54 nm / 40.4%
Detection type	Polydisperse	**Calibration constant**	197.01 ± 0.1 (nm/pixel)	**Camera type**	AVT Manta 319C
Cluster algorithm	Overridden	**Processing date**	2026-02-03 15:47:17	**Volume**	2.10×10⁻⁶ (mL)
Feature radius	40 pixels	**Total/proc. frames**	7500 / 7500	**Software/firmware**	2.61 / 1.3.5
Tracking starts on	1 frame	**Total/in range counts**	4689 / 4352	**Serial number**	0052

C. The overlay of data A (new protocol) and data B (old protocol)

HORIBA Scientific

ViewSizer 3000 - Nanoparticle Size Analyzer

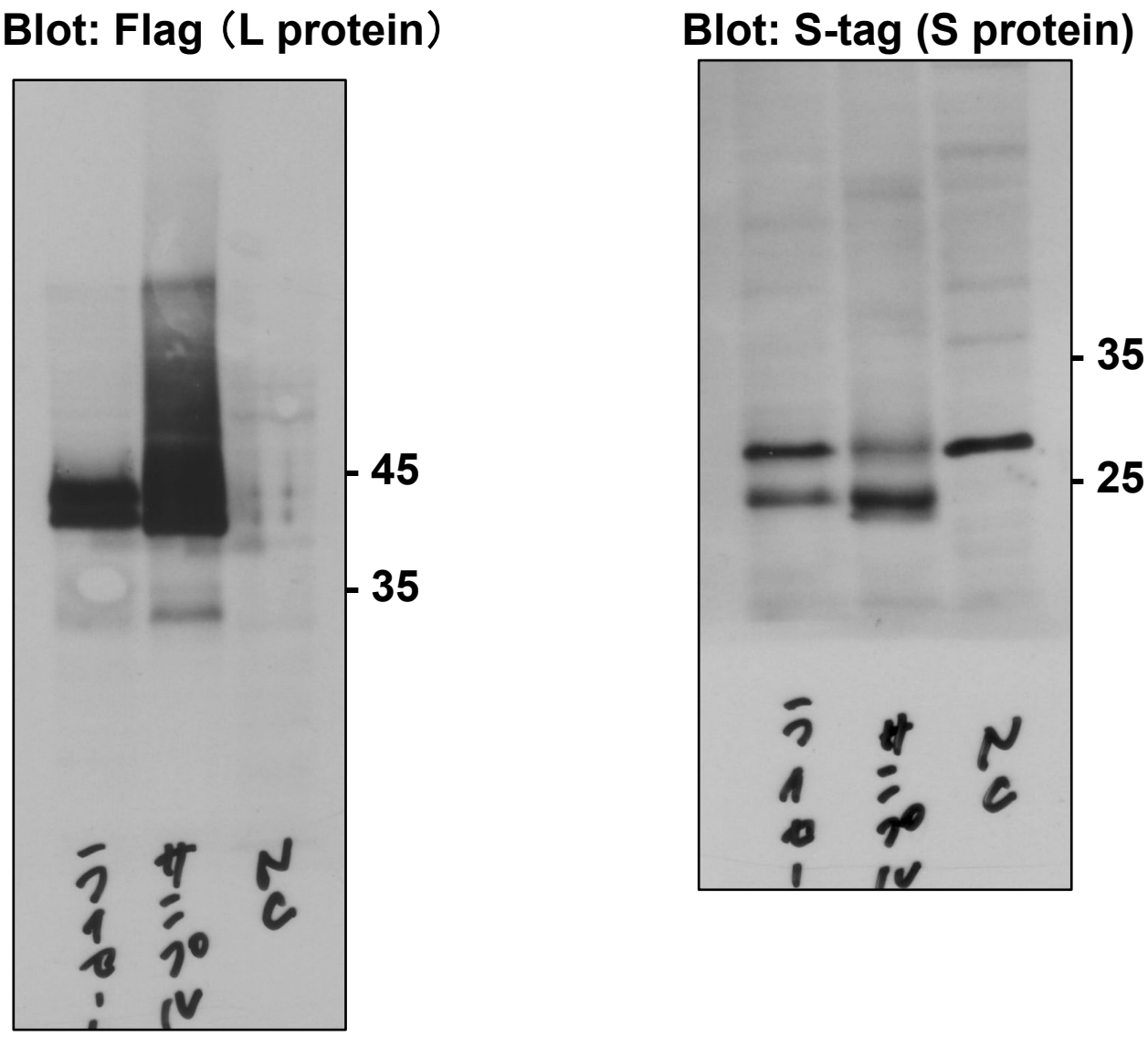


Sample	Color	Mode	Total counts	Mode (nm)	D50 (nm)	D10 (nm)	D90 (nm)	Span (nm)	Concentration (particles/mL)
sakai_method		MPTA	4352	112.50	125.08	74.54	206.20	1.053	8.29×10⁹
takayama_method_x100		MPTA	3190	87.44	126.77	72.83	220.67	1.166	6.08×10⁹
supernatant		MPTA	2764	127.56	128.14	77.26	212.40	1.055	5.26×10⁹
		Average	3435.33	109.17	126.66	74.88	213.09	1.09	6.54×10⁹
		CoV (%)	23.93	18.56	1.21	2.98	3.41	5.93	23.93

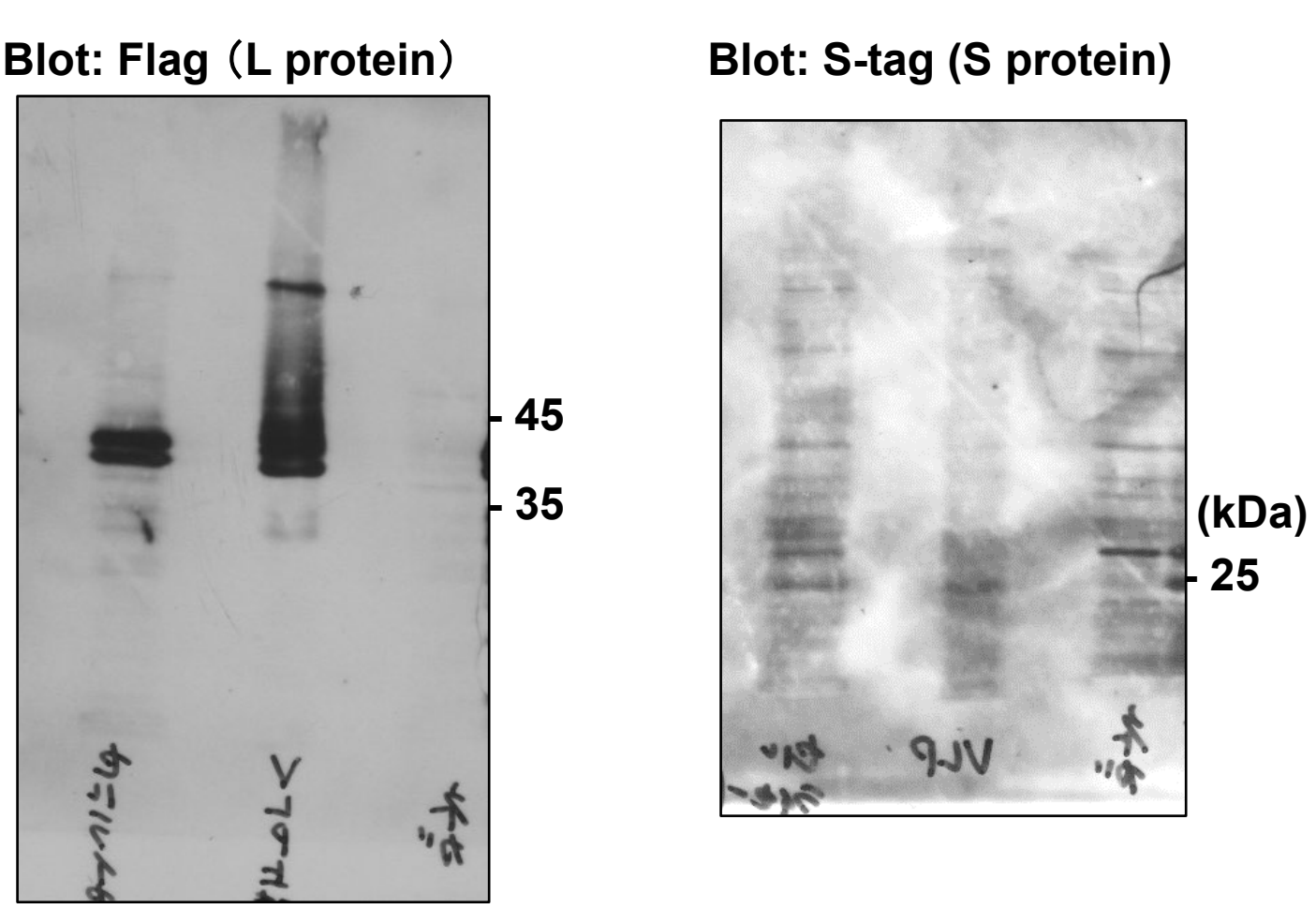
Supplementary Figure S2

Original Data of Fig. 1A and 1B

A

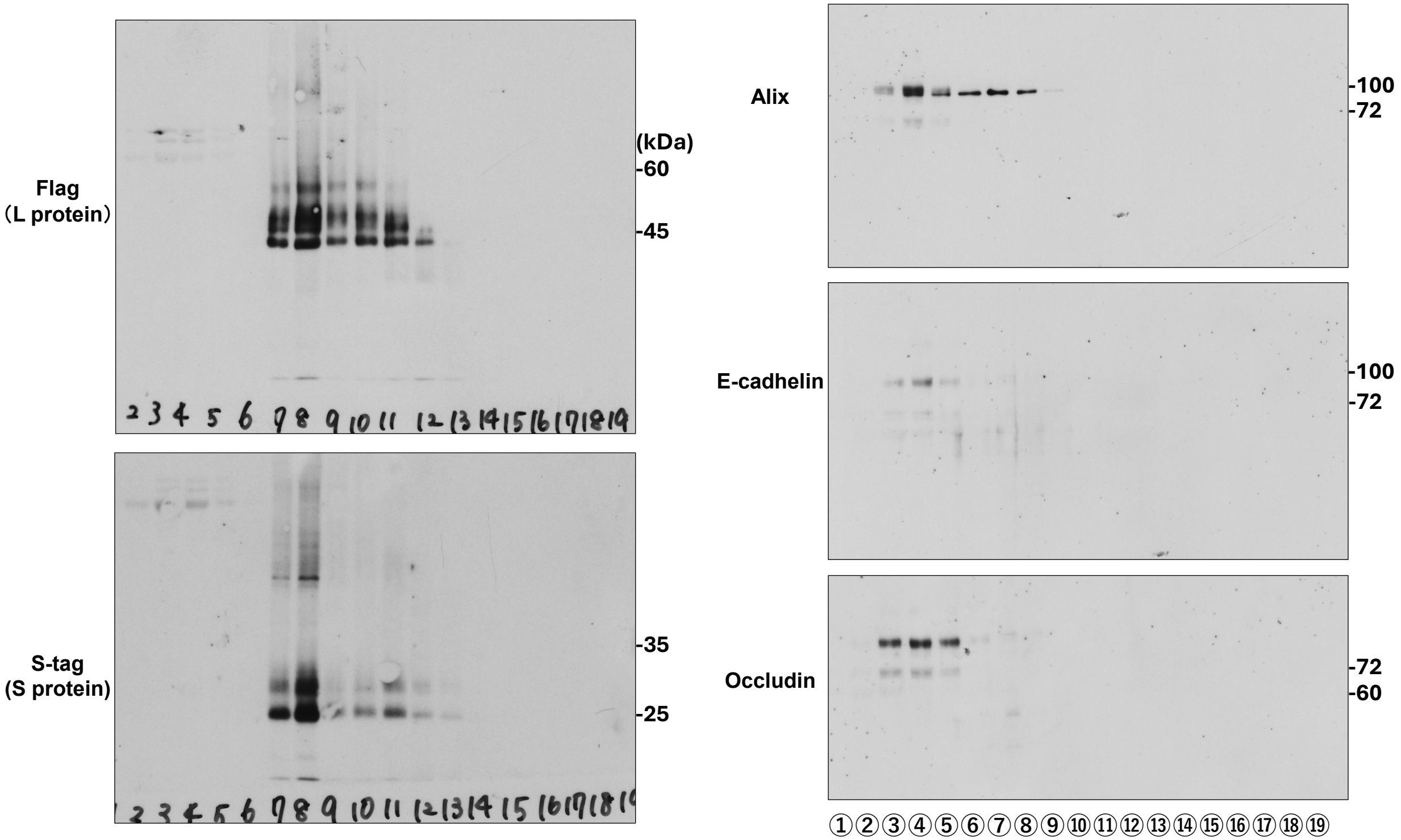


B



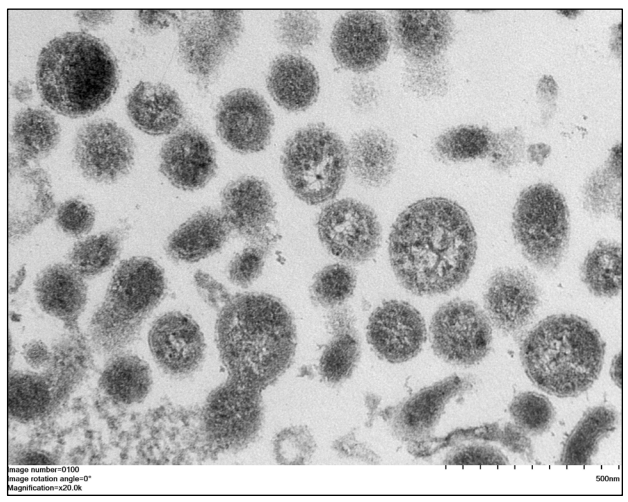
Supplementary Figure S3

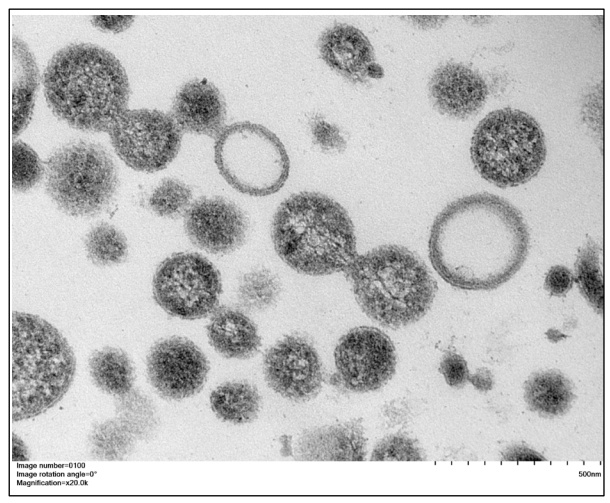
Original Data of Fig. 2B



Supplementary Figure S4

Original Data of Fig. 2C





Supplementary Figure S5

Raw data of Particle tracking analysis (PTA) using ViewSizer 3000 in Fig 3 and Fig 5

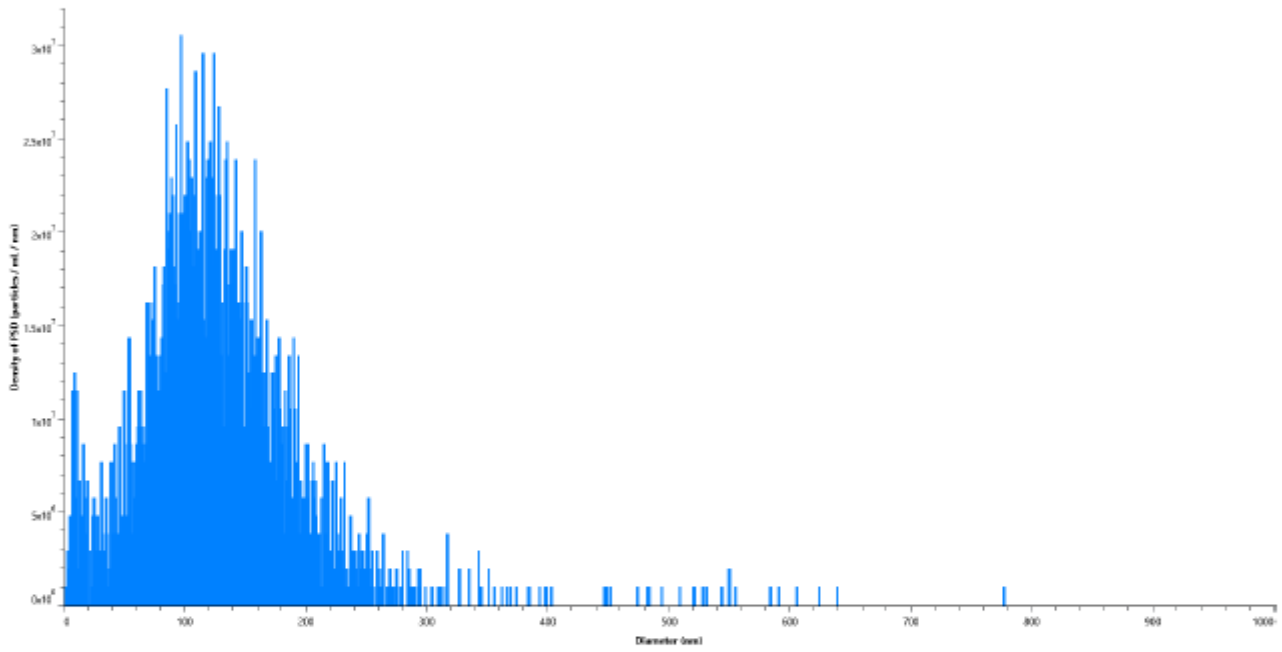
Particle concentration and particle size of VLSV

HORIBA Scientific

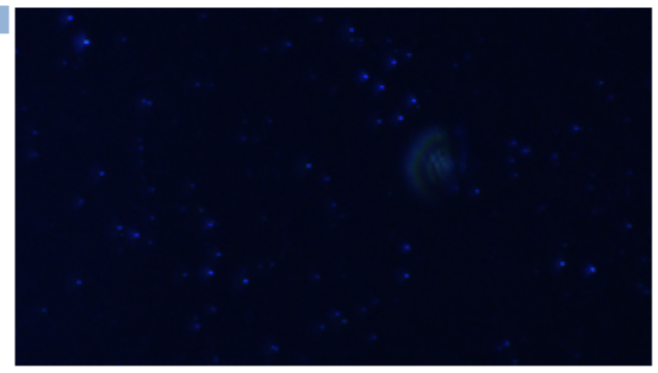
ViewSizer 3000 - Nanoparticle Size Analyzer

Sample information					
Sample name	#7-9	**Analyst**		**Recording date**	2026-03-19 14:22:05
Method	20260319_MPTA	**Sample type**	Sample		
Sample dilution	50	**Sample amount**	1 mg	**Density**	2 g/cm³
Blank name	N/A	**Blank concentration**	0.00×10¹ particles/mL		
File path	D:\HORIBA\VU-3000\Data\20260319_demo\#7-9\#7-9_20260319_142821.xml				
Blank file path	N/A				
Description	None				

Acquisition information		Multispectral Particle Tracking Analysis (MPTA)					
Blue laser (445 nm)	150 mW	**Stirring time**	3 s	**Focus**	10.309 mm	**Dispersant**	PBS
Green laser (520 nm)	12 mW	**Stirring speed**	1400 rpm	**Gain**	32 dB	**Viscosity**	0.9744 (mPa·s)
Red laser (635 nm)	8 mW	**Wait time**	2 s	**Exposure**	32 ms	**Refractive index**	1.34
Pulse duration (Blue)	32 ms	**Temperature**	22 °C	**Frame rate**	30 fps		
Pulse duration (Green)	32 ms	**Filter type**	None	**Frames/video**	300		
Pulse duration (Red)	32 ms	**Wavelength**	N/A	**Videos count**	25		



Parameter	Result
Mean	136.18 nm
Mode	97.50 nm
D10	75.41 nm
D50	125.89 nm
D90	206.03 nm
Span	1.038 nm
Concentration (sample)	2.49×10⁹ particles/mL
Concentration (minus blank)	2.49×10⁹ particles/mL
Integration range	50 - 500 nm
Chart type	ConstantBinsTable
Volume calibration	None



Video processing information					
Detection threshold	2	**Drift correction**	0%	**Std. dev./CoV**	57 nm / 41.5%
Detection type	Polydisperse	**Calibration constant**	197.01 ± 0.1 (nm/pixel)	**Camera type**	AVT Manta 319C
Cluster algorithm	Overridden	**Processing date**	2026-03-19 14:28:22	**Volume**	2.10×10⁻⁶ (mL)
Feature radius	40 pixels	**Total/proc. frames**	7500 / 7500	**Software/firmware**	2.61 / 1.3.5
Tracking starts on	1 frame	**Total/in range counts**	2875 / 2618	**Serial number**	0052