Supporting Information

Rapid and Ultrasensitive Fluorescent Screening of Extracellular Vesicles Using Dual-Aptamers Recognition-triggered RCA

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Experiment section

Materials and Reagents. All of the oligonucleotide sequences listed in **Table S1** were produced and refined through HPLC by Sangon Biotech Co., Ltd. (Shanghai, China). Tetramethylethylenediamine (TEMED) and ammonium persulfate (AP) were acquired from Sangon Biotech Co., Ltd. (Shanghai, China). Human malignant melanoma cells (A375), Fetal bovine serum (FBS), DMEM basic medium, trypsin, 10×TBE buffer, DNA marker, and Gel Red were received from both Thermo Fisher Scientific (Shanghai, China) and Procell Life Science & Technology Co., Ltd. (Wuhan, China). The enzymes and buffers utilized in the experiment, including Phi29 DNA polymerase, T4 DNA ligase, Escherichia coli exonuclease I (Exo I), Escherichia coli exonuclease III (Exo III), dNTPs, Phi29 buffer, PBS buffer and T4 buffer, were all ordered from New England Biolabs (NEB). The exoEasy Maxi Kit was obtained from QIAGEN (Germany). Dimethyl sulfoxide (DMSO) was purchased from Damao Chemical Reagent Factory (Tianjin, China). Thioflavin T (ThT) was purchased from Sigma-Aldrich (Shanghai, China). Polyacrylamide gel kits and ammonium molybdate staining solution were procured from Solarbio Science & Technology Co., Ltd. (Beijing, China). All water used in the experimental procedures was deionized water (ddH_2O).

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Table S1. Oligonucleotide sequences used in this work.

Oligonucleotide name	sequence (5' to 3') description
Apt-L1	CACCCCACCTCGCTCCCGTGACACTAATGCTATTTTTTTTTT TTTTT*CACATGACC*CATACCGA
Apt-L2	CATAAGACT*GGTCATGTG*TTTTTTTTTTTTTTTTACGGGCCAC ATCAACTCATTGATAGACAATGCGTCCACTGCCCGT
PL	AGTCTTATGCAGCATGGAGGTTTTTCAGCATGGAGGTCGGT ATG
LP	CATAAGACTCATACCGA

The underlined portions of Apt-L1 and Apt-L2 in the table can form a complete ligation probe to bind with the PL chain, forming a complete rolling circle template; the italicized portion in Apt-L1 can complement the italicized portion in Apt-L2. The LP sequence is an integration of the underlined portions of Apt-L1 and Apt-L2.

Table S2. Comparison of different methods for EVs determination.

Methods	Detection Range (particles / mL)	Limit of Detection (particles / mL)	Reference
Fluorescent assay	2×10^4-2×10^9	2.9×10^3	[1]
Electrochemical assay	2.47×10^8-1.23×10^9	9.3×10^7	[2]
Colorimetric assay	1×10^5-1×10^7	4.7×10^4	[3]
Fluorescent assay	1.75×10^3-3.5×10^8	1.02×10^3	[4]
Fluorescent assay	10^4-10^8	1.4×10^3	[5]
Fluorescent assay	1×10^3-1×10^{10}	7.64×10^2	This work

References

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