Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Ultrasensitive quantification of extracellular vesicles through dual signal amplification for early diagnosis and prognosis of chronic obstructive pulmonary disease (COPD)

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Table S1. Sequences of oligonucleotides

Title	Sequence (5'-3')
Probe-1	CAC GCC GAA TCC TAG ACT ATT TTT ATA GTC TAG GAT TC**G** **ACT C**CC GTC TAC TCG CTG AAC TG
Probe-2	CAG TTC AGC GAG AGA CGG CCT TTT GGC CGT CTA TGC GTG AAC TGG GCC TCT AGG ATT CGG CGT G
Capture probe	ATA TAC ACC CCT ACT CGC TCC CGT GAC ACT AAT GCT ATT TTC AGT TCA GCG AGT AGA CGG AGT
F-Capture probe	**Cy3**-ATA TAC ACC CCT ACT CGC TCC CGT GAC ACT AAT GCT ATT TTC AGT TCA GCG AGT AGA CGG AGT-**BHQ-1**
F-Probe-1	**Cy3-**CAC GCC GAA TCC TAG ACT ATT TTT ATA GTC TAG GAT TC**G** **ACT C**CC GTC TAC TCG CTG AAC TG-**BHQ-1**

Experimental section

Extraction of EVs

Extraction protocol of EVs was from some of the former proposed methods. With A549 as supplier, EVs were extracted from cell culture medium. A549 cells were incubated in a medium containing RPMI-1640 medium and 10% fetal bovine. The mixture were incubated in a humidified atmosphere of 5% CO_2 at 37°C. before extraction of EVs, A549 cells were washed for 3 times by PBS buffer and then incubated for another additional 12 h. Through ultra-centrifugation, the obtained culture medium was standard differential centrifuged and the white flocculent precipitation is extracted EVs. Finally, transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA) were performed to characterize these isolated EVs: the concentration and size distribution of extracted exosomes were analyzed by NTA, which was performed using the Nanosight NS300 followed by protocol. TEM imaging was carried out using a JEM-1400Plus.

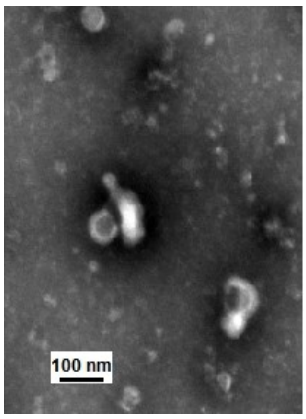

Figure S1. EVs extraction and feasibility of the probes for signal amplification. *(a) TEM result of the extracted EVs;*