**Supporting Information**

**Micro size exclusion chromatography combined with a multiplex protein profiling method for extracellular vesicle protein detection from small sample volumes**

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**Supplementary Figures and Tables**



**A**



**B**



**Figure S1. qPCR on serial dilution of TotalSeq Oligos.** (A) Ten-fold dilution of seven different TotalSeq Oligos were performed only the ligation and qPCR step. The oligos were either tested alone (left) or in a mixture (right). (B) Two dilution curves from same oligo alone or mixed sample were plotted in the same chart.



**Figure S2.** **Spike-in oligo into other oligo mixture.** Different concentration of PD-L1 oligo were spiked into an equal molar mixture of four other oligos (CD63, CD81, EGFR, Ctl Ig).

**Table S1. qPCR primer specify test**

|  |  |  |
| --- | --- | --- |
|  |  | Oligo Sample |
|  |  | **CD9** | **CD63** | **CD81** | **PD-L1** | **EpCAM** | **EGFR** | **Ctl Ig** |
| qPCRPrimer | **CD9** | 4.73 | 34.98 | 35.63 | 34.56 | 32.16 | 36.24 | 34.55 |
| **CD63** | N.D. | 16.61 | N.D. | N.D. | N.D. | N.D. | N.D. |
| **CD81** | N.D. | 37.81 | 14.59 | N.D. | N.D. | N.D. | N.D. |
| **PD-L1** | N.D. | N.D. | N.D. | 19.40 | N.D. | N.D. | N.D. |
| **EpCAM** | N.D. | N.D. | N.D. | N.D. | 26.68 | N.D. | N.D. |
| **EGFR** | N.D. | 32.92 | 37.17 | 39.19 | N.D. | 14.00 | N.D. |
| **Ctl Ig** | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. | 4.80 |

 0.5 nM of each oligo sample was tested with all other qPCR primers, Ct value of qPCR was shown in the table. N.D. = not detectable



**Figure S3. TotalSeq Protocol test with Biotin-BSA and Streptavidin-Oligo.** Biotinylated BSA was blotted on the strip as analyte, and probe by oligo conjugated streptavidin. Ct of qPCR was plotted to BSA amount.



**B**

**A**



**Figure S4.** **Dot blot of BSA.** (A) Serial dilution of biotinylated BSA were blotted on the NC membrane and tested under standard protocol with streptavidin-HRP. The membrane was exposed for 4s, 30s or 5 minutes. (B) The plot shows optical intensity to protein amount.



**Figure S5.** **Ct value comparison of cDNA from isolated RNA or direct reverse transcription.** Average Ct values of RNAs isolated from cell, RNAs isolated from supernatant and supernatant directly into cDNA were clustered into 3 groups. ΔCt was the difference compared with RNAs isolated from cell.