Electronic Supplementary Material (ESI) for Analyst.

Supplementary information

Enhanced Paper-based ELISA for Simultaneous EVs/exosome Isolation and Detection using Streptavidin Agarose-based Immobilization

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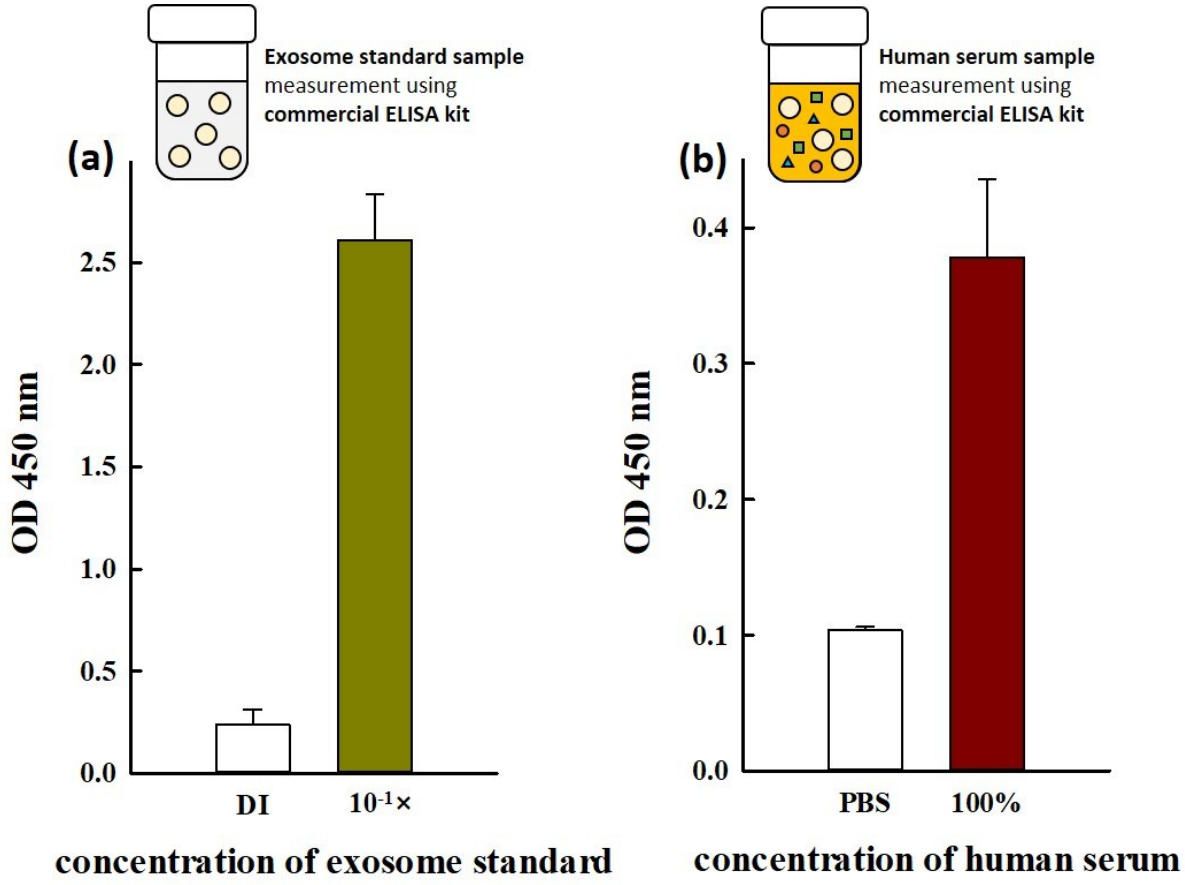

Figure S1. Commercial EVs/exosome verified using commercial exosome ELISA kit. (a) Measurement of exosome standard sample and (b) measurement of human serum sample.

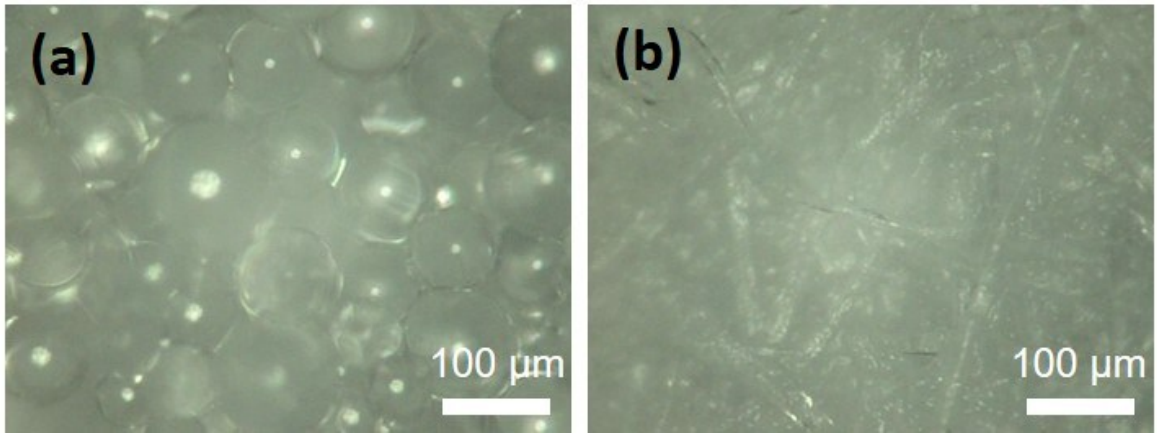

Figure S2. Optical images of agarose beads on the (a) top side and (b) bottom side of the paper well after loading agarose gel on top of the hydrophilic paper wells. The streptavidin agarose resin used in this study contains 50% aqueous slurry containing 0.02% sodium azide as a preservative. We used 5 µl of streptavidin agarose resin; accordingly, 2.5 µl of agarose beads are expected. We optimally designed the volume capacity of the hydrophilic paper well (reservoir) as 3.5 µl, which is suitable for agarose beads.

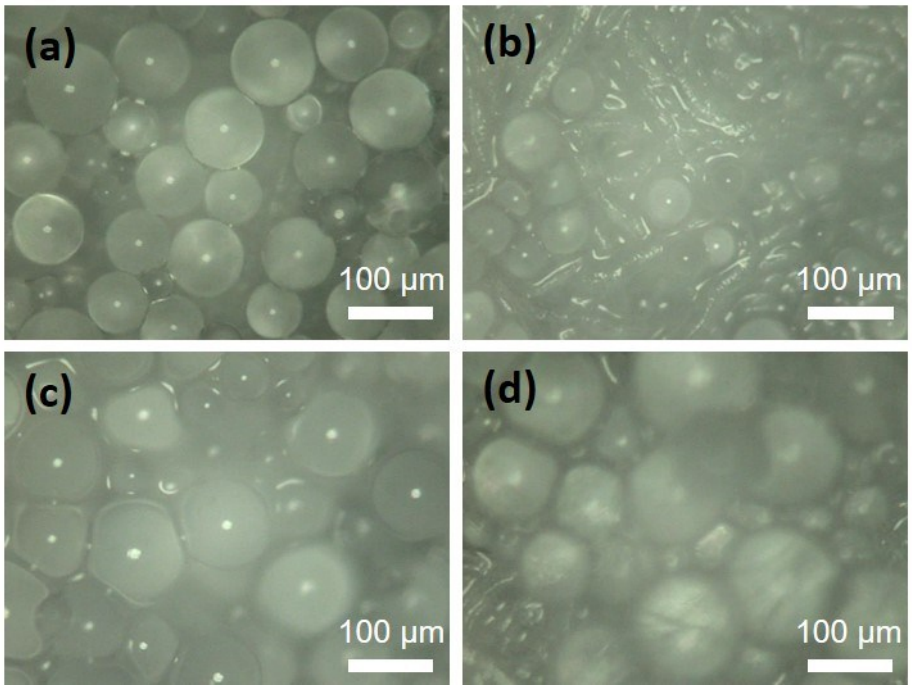

Figure S3. Optical images of agarose beads on the surface of paper well (reservoir). (a-b) Optical images of agarose beads (a) before washing step and (b) after mechanical washing step. Gentle washing was conducted using the mechanical shaker with 20 μl of phosphate-buffered saline (PBS), which reveals the losses of agarose beads from paper wells. (c-d) Optical images of agarose beads (c) before and (d) after washing step via the absorbent pad. For this purpose, we used the absorbent pad on the bottom side of paper wells and added washing buffer twice (20 μl of PBS) at the top side. The added buffer washed out nonspecific ones and was then absorbed through the absorbent pad; this significantly reduced the losses of agarose beads during the washing step.

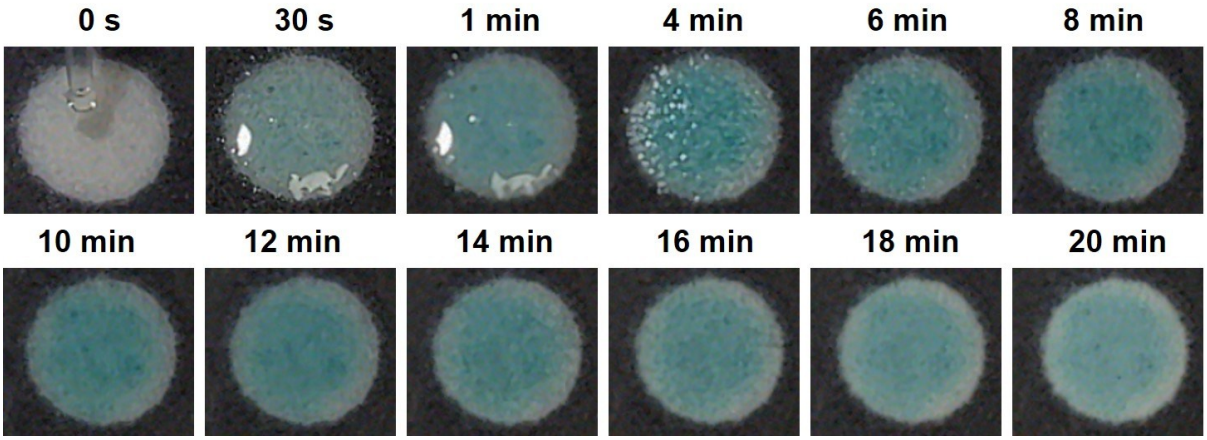

Figure S4. Optical images after injecting TMB for p-ELISA. With the captured ES (10× diluted ES sample), we followed the assay sequence shown in Fig. 2 and then added TMB. The color intensity of TMB gradually becomes saturated after 8 min. All measurements were performed after 20 min.

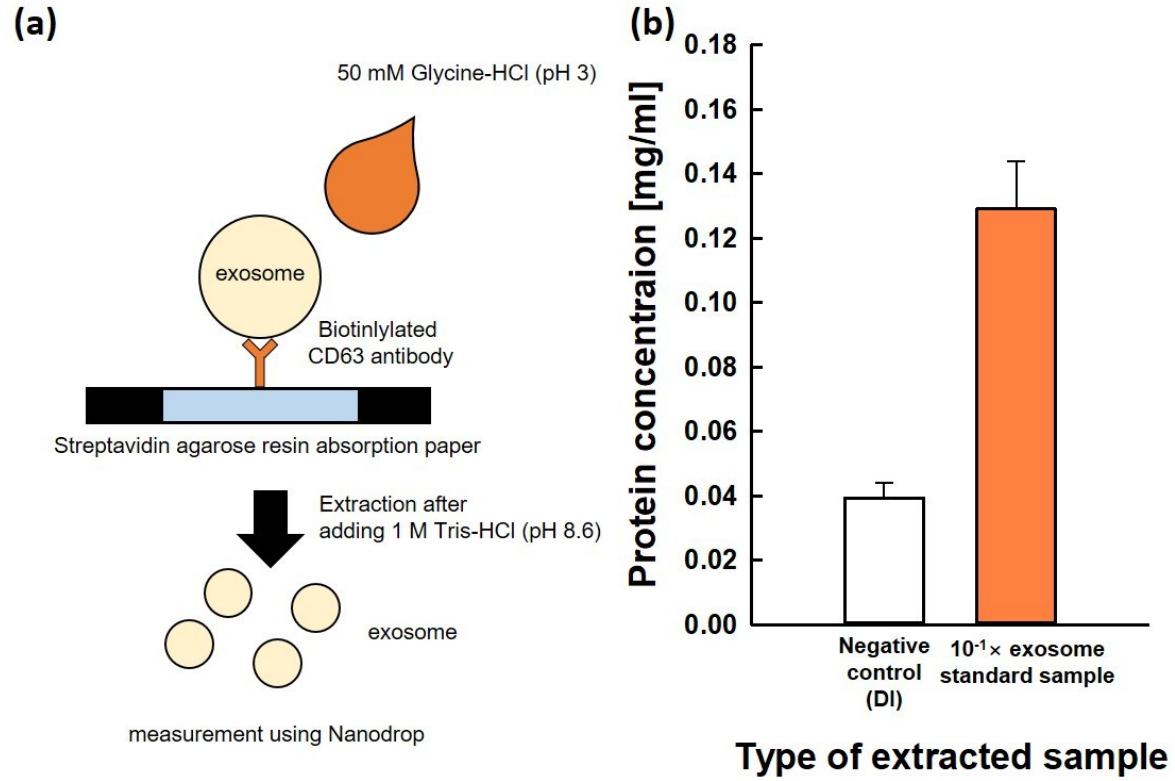

Figure S5. Extraction of exosome using a Glycine-HCl (pH3) for downstream analysis. (a) Scheme of exosome extraction from paper materials and (b) Protein concentration of extracted samples from DI water (negative control) and 10^{-1} X exosome standard samples. The measurement was performed using a Nanodrop^{R} (Thermo Fisher Scientific).