Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Integrated phase separation in microliter droplets for ultratrace-enriching biomarker analysis

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KEYWORDS: Minipillar array, Aqueous two-phase system, Ultratrace, Enrichment, Fluorescence.

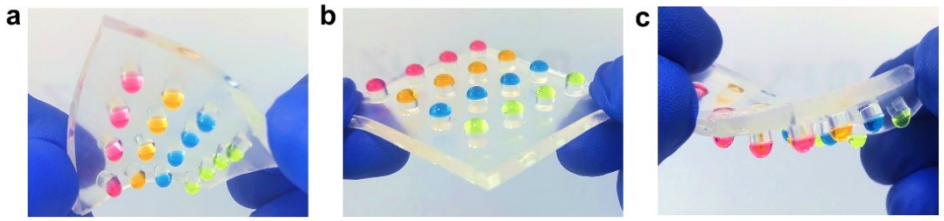

Figure S1. Performance of the PDMS minipillar array. Whether (a) bent, (b) stretched, or (c) twisted, the droplets can be securely anchored to the minipillar array.

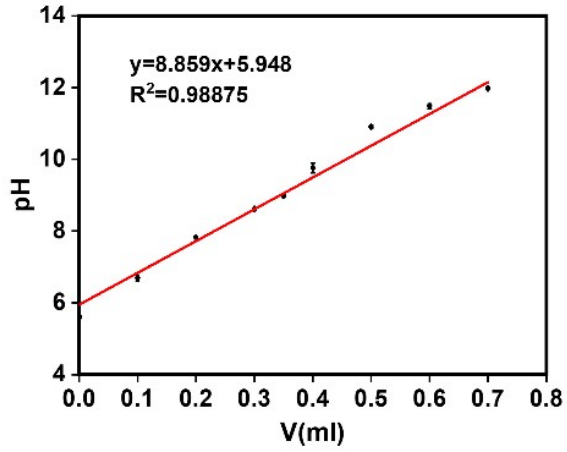

Figure S2. pH allocation of PEG solution. The PEG solution is not equipped with a standard pH solution as its enrichment effect is significantly inferior to that of the NaOH solution. Additionally, the quantity of the NaOH solution added during the solution preparation is directly proportional to the pH value.

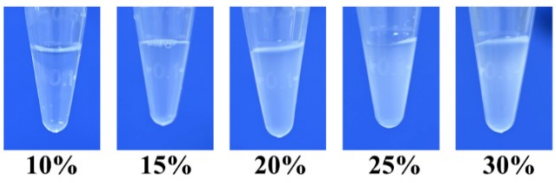

Figure S3. Image of the solution obtained after mixing PEG solutions of varying mass fractions with DEX solution. As depicted in the figure, it is evident that when the volume ratio is 10:1, the mixture of 10% and 15% PEG solution with 30% DEX solution remains clear and transparent, preventing the separation of the two phases.

However, when PEG with a mass fraction exceeding 15% is mixed with 30% DEX solution, the resulting mixture becomes cloudy, allowing for the separation of the two phases.

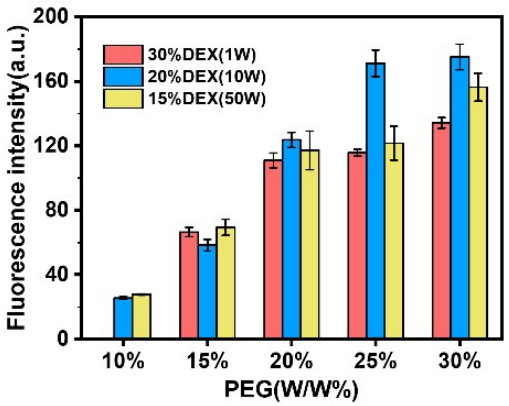

Figure S4. The fluorescence intensity map of DEX and PEG enriched miRNA with varying mass fraction and molecular weight. The fluorescence intensity of 30% DEX (10000), 20% DEX (100000), and 15% DEX (500000) was measured after enrichment through mixing with 10%, 15%, 20%, 25%, and 30% PEG (8000). Volume ratios=1:15, pH=9.0.

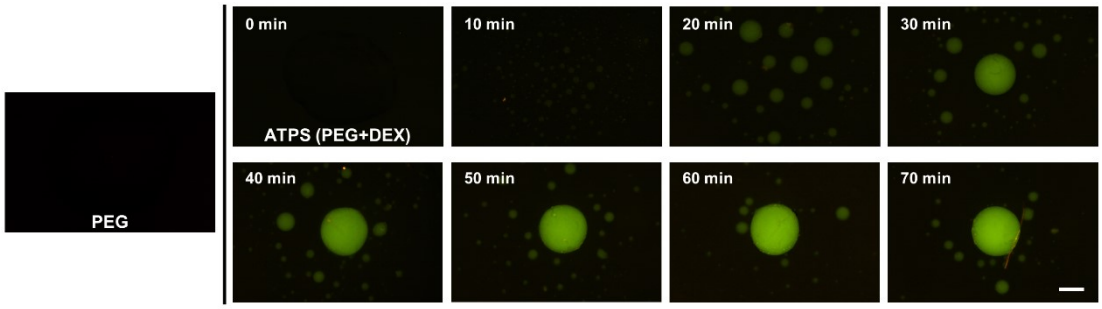

Figure S5. The volume of the DEX-rich phase increased gradually during the static phase separation, and the fluorescence intensity also increased gradually. Scale bar: 500 μm.

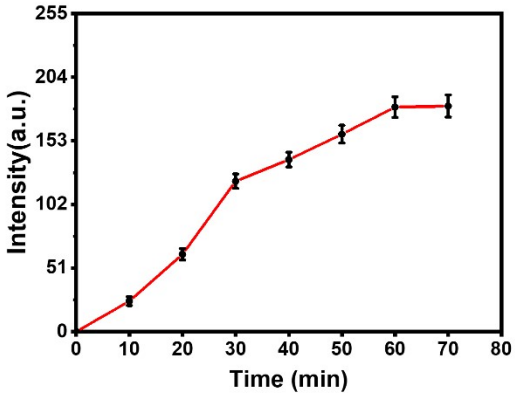

Figure S6. Fluorescence intensity at different time points.

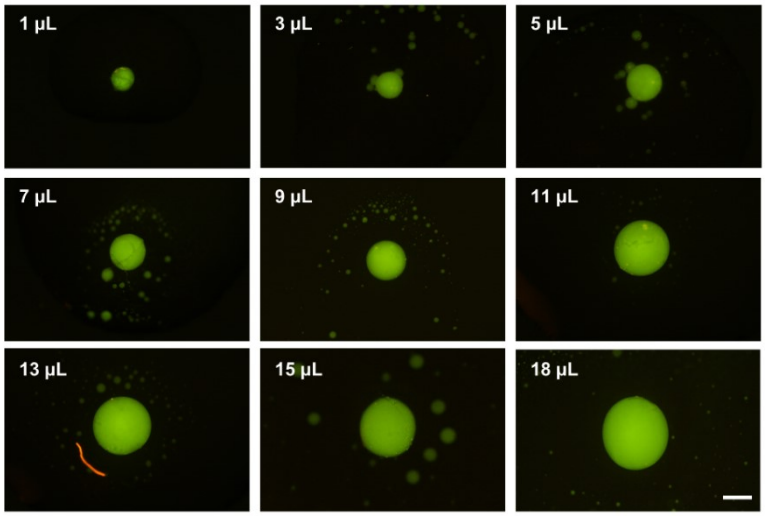

Figure S7. Fluorescence patterns obtained by static phase separation of different volumes of ATPS droplets placed on a minipillar. Scale bar: 500 μm

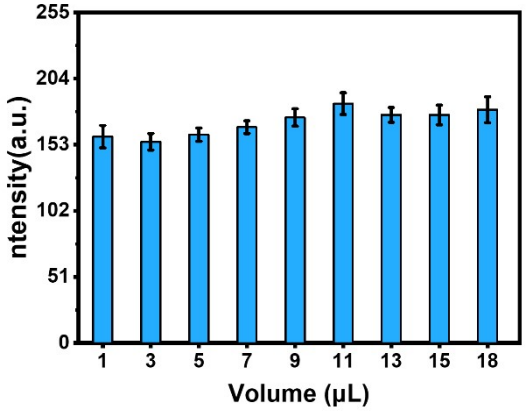

Figure S8. Fluorescence intensity of the DEX-rich phase after phasing of droplets of different volumes of ATPS.

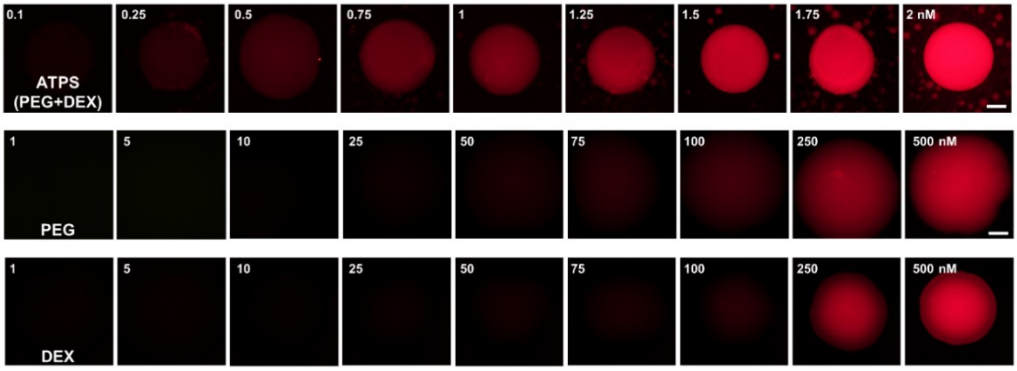

Figure S9. The red fluorescence images with different concentrations of miRNA-141 in ATPS microdroplet, single-phase PEG and DEX systems. Scale bar: 200 μm (ATPS), 500 μm (PEG), 500 μm (DEX).

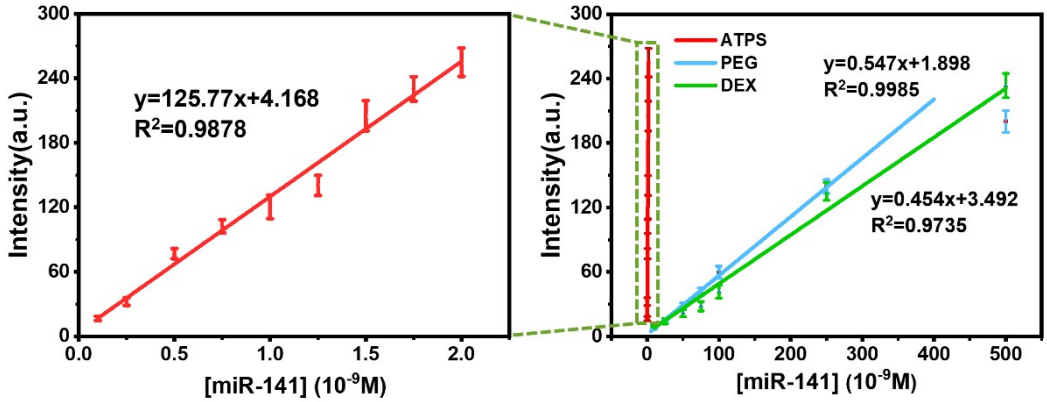

Figure S10. A linear correlation between the intensity of the red fluorescence signal and the concentration of miRNA-141 in ATPS microdroplet and, single-phase PEG and DEX systems.

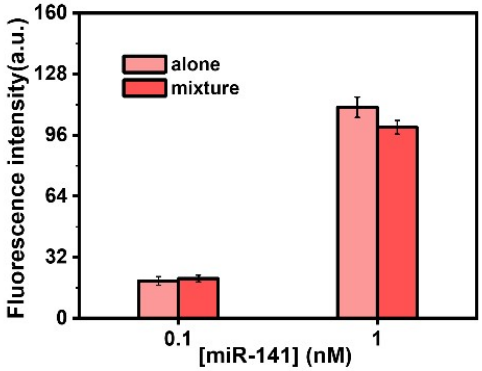

Figure S11. Comparison of fluorescence intensity between mixed detection of miR-

141 and single detection.

Table. S1. Comparison of the LOD and sample volume with similar techniques.

Detection target	Method	Sample volume	Detection time	LOD	Reference
miRNA-21	Fluorescence	50 μL	1 h	1 nM	[1]
miRNA-21	Fluorescence	50 μL	40 min	1 pM	[2]
miRNA-21	Fluorescence	200 μL	1 h	2 pM	[3]
miRNA-155	Fluorescence	-	2 h	100 pM	[4]
miRNA-21/miRNA-141	Fluorescence	18 μL	1 h	156 pM /50.3 pM	this work

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