Supplementary material

Table 1. Oligonucleotide sequences used in this work.

Title	Sequences (5' to 3')
Target miRNA	UGA GGU AGU AAG UUG UAU UGU
Let-7a	UGA GGU AGU AGG UUG UAU AGU U
miRNA-141	UAA CAC UGU CUG GUA AAG AUG G
S1	CCC TAA CCC TAA CCC TAA CCC T GCT GAG G TGG TAA CGG AGG TGG TTT CAA GTC GTC CAG TTG AAA
S2	TTT CAA CTG GAC GAC TTG AAA AAC AAT ACA ACT TAC TAC CTC A GCT GAG G TTT CAA CTG GAC GAC TTG AAA TTT CCT CTG GAC GAC TTG AAA AAC AAT ACA ACT TAC TAC CTC A TTT CAA GTC GTC CAG TTG AAA
S3	CCA CCT CCG TTA CCA AAC AAT ACA ACT TAC TAC CTC A GCT GAG G TTT CAA GTC GTC CAG TTG AAA

Table S2. A brief comparison of the method with former ones.

Title	Mechanism	LOD	Duration	Detection mode	One-pot	Ref
The method	Self-priming cyclic amplification	0.82 fM	100 min	In *vitro*	Yes	
RACE	RCA	90 fM	150 min	In *vitro*	No	[1]
vcPeLa	PER+ LAMP	0.31 fM	120 min	In *vitro*	Yes	[2]
FISH-Cas12a	Cas12a	0.42 pM	150 min	In *vitro*	No	[3]
Y-shaped probe mediated	EXPAR	0.58 nM	60 min	In *vitro* and in *vivo*	No	[4]

Notes: EXPAR, exponential amplification reaction; PER, primer exchange reaction; RCA, rolling circle amplification; LOD, limit of detection

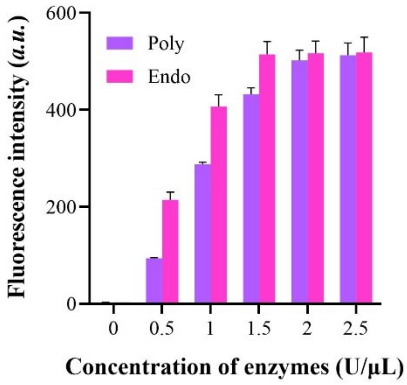

Figure S1. Fluorescence intensity of the method when detecting 1pM target miRNA with different enzyme concentration.

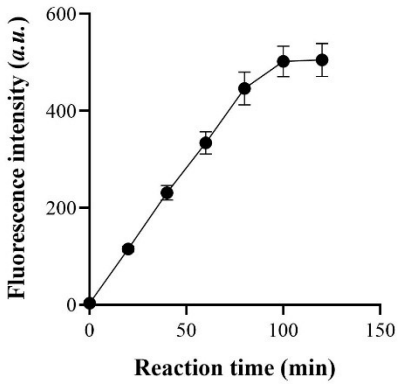

Figure S2. Fluorescence intensity of the method when detecting 1pM target miRNA with reaction time.

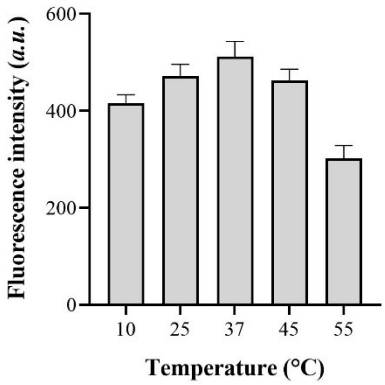

Figure S3. Fluorescence intensity of the method when detecting 1pM target miRNA with different temperature.

Supplemented experimental section

qRT-PCR protocol

Total RNA was extracted from serum samples using TRIzol LS Reagent (Thermo

Fisher Scientific) according to the manufacturer's instructions. Reverse transcription was performed with a stem-loop RT primer specific to the target miRNA using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems). The resulting cDNA was then amplified in a QuantStudio 5 Real-Time PCR System using TaqMan Universal PCR Master Mix and a specific TaqMan MicroRNA Assay. The thermal cycling conditions were: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. Each sample was run in triplicate, and relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method with appropriate endogenous controls.

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of The Third People's Hospital of Cangnan County (IRB No. TPHCC-2024-032). Written informed consent was obtained from the parents or legal guardians of all participating children.

References:

1. Wang, R.; Zhao, X.; Chen, X.; Qiu, X.; Qing, G.; Zhang, H.; Zhang, L.; Hu, X.; He, Z.; Zhong, D.; Wang, Y.; Luo, Y., Rolling Circular Amplification (RCA)-Assisted CRISPR/Cas9 Cleavage (RACE) for Highly Specific Detection of Multiple Extracellular Vesicle MicroRNAs. *Anal. Chem.* **2020,** *92* (2), 2176-2185.

2. Liu, X.; Peng, H.; Ye, X.; Zhang, X.; Xu, G.; Zhao, X., A Versatile Colorimetric Diagnostic Platform Based on Primer Exchange Reaction Cascades Driven Loop-Mediated Isothermal Amplification. *Anal. Chem.* **2025,** *97* (16), 9000–9007.

3. Liu, J.; Li, X.; Zhou, D.; Yang, H.; Liu, M.; Su, K.; Yan, Y., Fluid interface-constrained split crRNA-Cas12a for rapid and high sensitive miRNA detection. *Microchem. J.* **2025,** *219*.

4. Du, Y.; Sun, R.; Hao, S.; Yang, F.; Hu, Q.; Xue, R., Y-shaped nanoprobe-based DNA microarray for rapid detection of multiplex miRNAs with single-nucleotide specificity. *Microchem. J.* **2026,** *222*.