Supplementary Information

MNAzyme-Assisted Nucleic Acid Lateral Flow Assay for Cost-Effective, On-Site Mercury Detection

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Table S1. Oligonucleotide sequences used in this study.

Name	Sequence (5′ to 3′)
Substrate	CTC TAG CAT GGA CC**rG rU**CT GAC TGT ATC TC
M1 DNA	CAG TCA GAG GCT AGC T<u>TA TTG TGT TGC TGC</u>
M2 DNA	<u>GCA GCT TCT CTT TA</u>ACA ACG AGG TCC ATG
Tag DNA	TTT TTT TCG GTC CAT GCT AGA GAA AAA AA
Capture DNA in the test line	GAG ATA CAG TCA GA
Capture DNA in the control line	CTC TAG CAT GGA CCG
Cleaved substrate (I)	CTC TAG CAT GGA CCG
Cleaved substrate (II)	TCT GAC TGT ATC TC

* Bold letters indicate ribonucleotide and cleavage sites. Red letters indicate the catalytic core of the MNAzyme. Underlined letters indicate the Hg^{2+} recognition sequences.

Table S2. Comparison of this method with the previous colorimetric methods for Hg^{2+}.

Key detection components	Limit of Detection (ppb)	Detection Time (min)	Reference
3D-printed rolling circle amplification chip	2.2	60	[1]
G-quadruplex based DNAzyme	10	130	[2]
G-quadruplex based DNAzyme	20	140	[3]
Phosphorothioate based DNAzyme	10	215	[4]
MNAzyme-assisted NALFA	1.874	120	This work
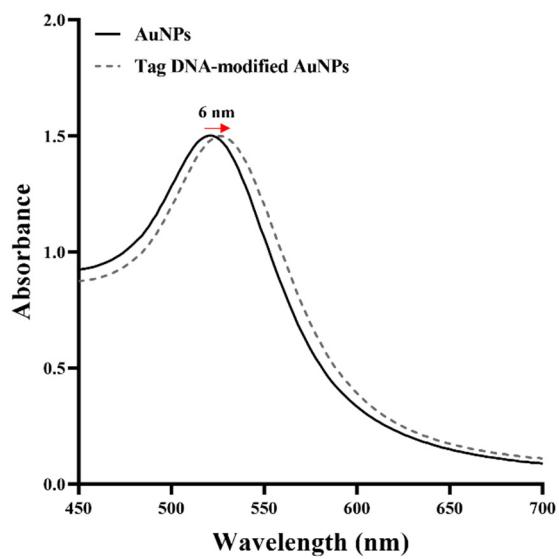
Figure S1. Absorbance spectra of AuNPs (solid) and Tag DNA-modified AuNPs (dotted).

Side view

23 mm

Absorbent pad

25 mm

NC membrane

Backing Card

Top view

45 mm

● Control line

● Test line

3 mm
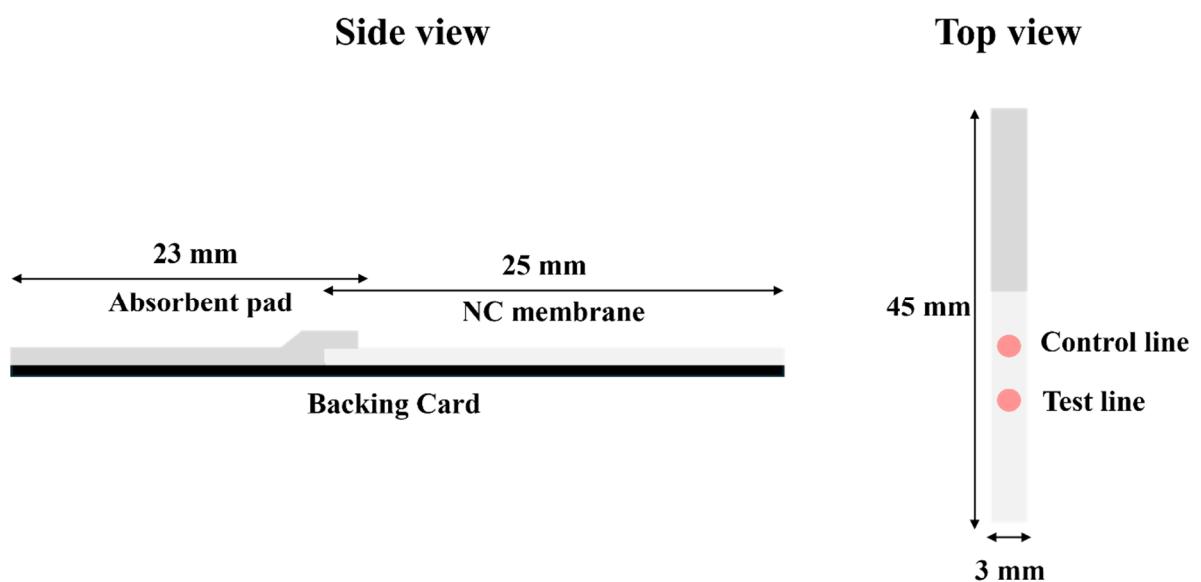
**Figure S2**. Schematic illustration of the NALFA strip.
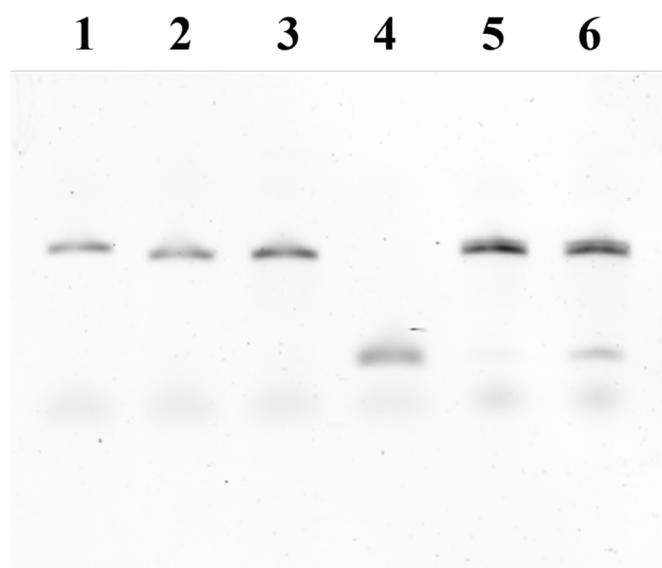
Figure S3. Images of the Urea-PAGE gel. Lanes 1-4 are M1 DNA, M2 DNA, the substrate, and the cleaved substrates (I and II; Table S1), respectively, and lanes 5 and 6 are the reaction products where all detection components (M1 DNA, M2 DNA, and substrate) are present without and with Hg^{2+} (100 ppb), respectively.
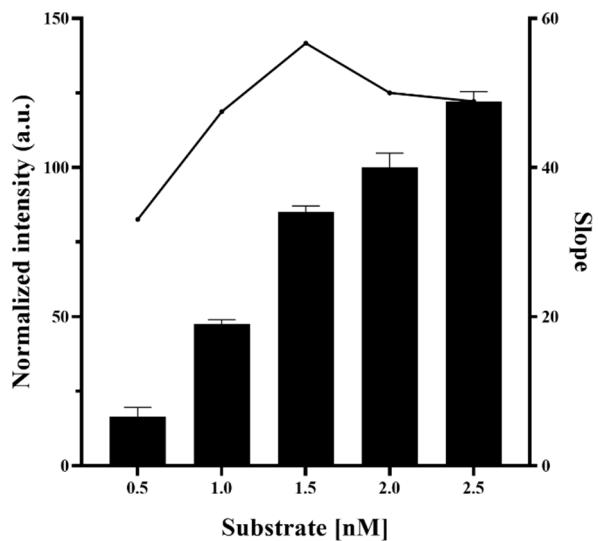
Figure S4. Normalized intensity at different substrate concentrations in the NALFA system. The slope was calculated by dividing the increase of the normalized intensity by each substrate concentration. Error bars represent the standard deviation from three independent experiments.

References

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