

## Supplementary information

### Characterizations

The immobilization of mAb-COT onto the AuNP was monitored using a UV-vis spectrophotometer. The maximum absorption wavelength of the AuNP was 523 nm, while the wavelength of the mAb-COT-AuNP was slightly shifted to 526 nm (Figure 1S). The minor shift was caused by a change in the refractive index, which caused the layer mAb-COT that was conjugated onto the AuNP.

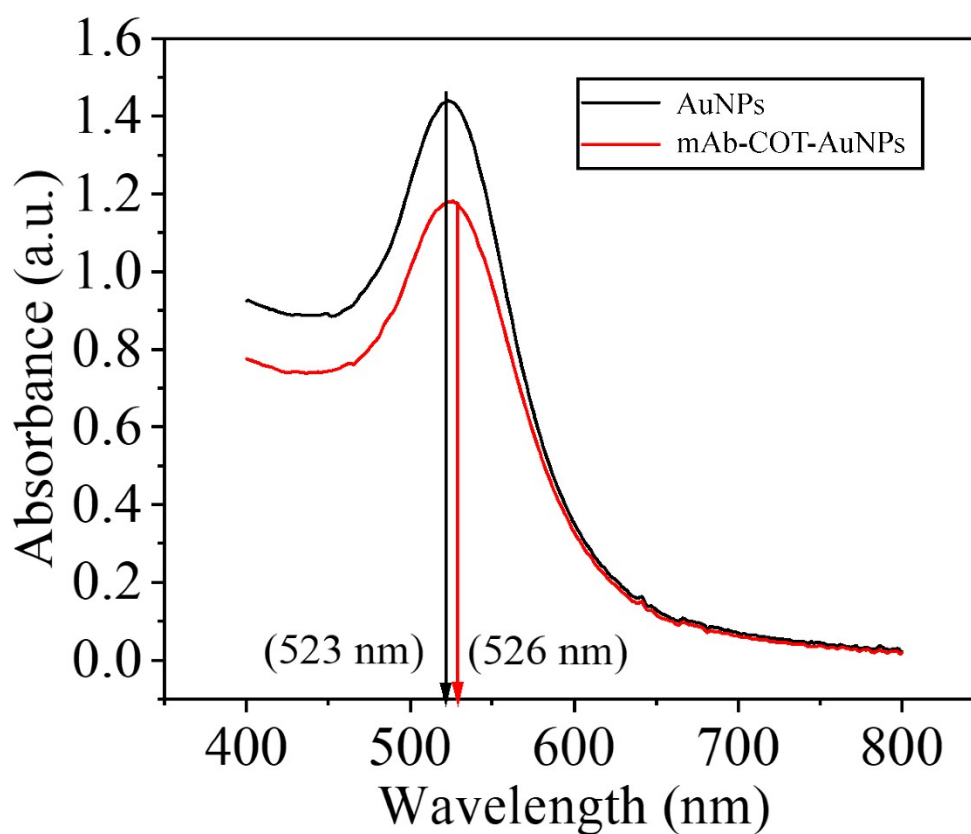


Figure 1S. Absorption spectra of AuNP (black line) and mAb-COT-AuNPs (red line).

Table S1. Comparison of cotinine screening results in pooled urine samples using the ELISA assay and the developed LFIA.

Spiked cotinine (ng/mL)	Found	
	ELISA (ng/mL)	LFIA
0	3.1	Negative
25	29.5	Negative
50	68.9	Positive
100	81.7	Positive
150	155.1	Positive

200	216.2	Positive
300	357.4	Positive
500	423.6	Positive
1000	900.0	Positive