

## **Dual-Enhancement Lateral Flow Immunoassay via Gold-Decorated Ferritin Nanoprobes and SpyTag/SpyCatcher Site-Directed Conjugation**

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# 1. Effect of addition order on ST-HFn@Au assembly

According to Table S1, add PB buffer and 0.2% AuNPs into a 1.5 mL centrifuge tube, mix thoroughly, then quickly add 50  $\mu$  L of 2 mg/mL ST-HFn and mix immediately.

**Table S1.** Preparation of ST-HFn@Au at different dilutions (normal addition)

Dilution	PB buffer( $\mu$ L)	0.2%AuNPs( $\mu$ L)	2 mg/mL ST-HFn ( $\mu$ L)
2 $\times$	200	200	50
4 $\times$	400	200	50
6 $\times$	600	200	50
8 $\times$	800	200	50
10 $\times$	1000	200	50

According to Table S2, add PB buffer and 50  $\mu$  L of 2 mg/mL ST-HFn into a 1.5 mL centrifuge tube, mix thoroughly, then quickly add 200  $\mu$  L of 0.2% AuNPs and mix immediately.

**Table S2.** Preparation of ST-HFn@Au at different dilutions (added in reverse order)

Dilution	PB buffer( $\mu$ L)	0.2%AuNPs( $\mu$ L)	2 mg/mL ST-HFn ( $\mu$ L)
2 $\times$	200	50	200
4 $\times$	400	50	200
6 $\times$	600	50	200
8 $\times$	800	50	200
10 $\times$	1000	50	200

ST-HFn@Au prepared under different dilutions and addition orders, as well as AuNPs, were taken and diluted to the same concentration (calculated as Au). Then, 200  $\mu$  L of each diluted sample was added to a 96-well plate, with PB buffer serving as a blank control. The 96-well plate was photographed, and the images were processed and subjected to grayscale analysis using Image J software.

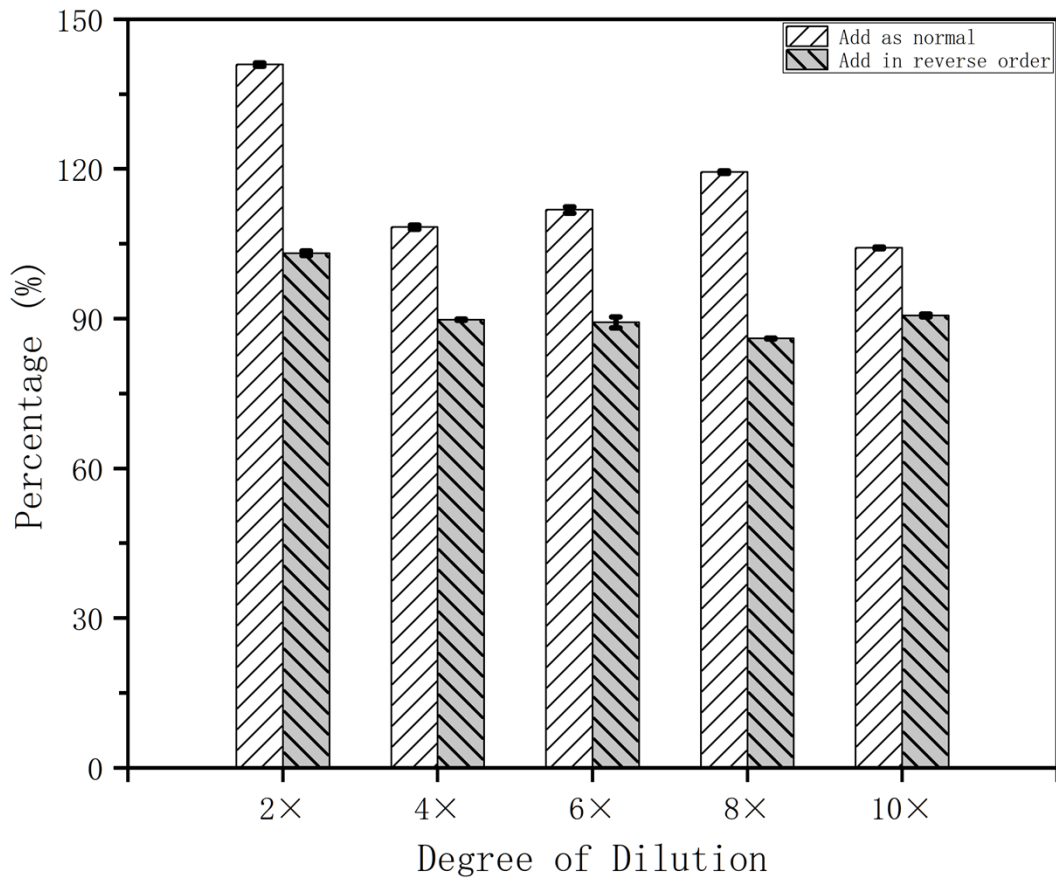


Figure S1. Effect of addition order

## 2. Precision of detection

Intra-batch consistency evaluation: Test strips from the same batch were used to detect hCG at concentrations of 2.5, 10, and 20 mIU/mL. The corresponding T/C values were measured, and the coefficient of variation (CV) was calculated.

Table S3. Intra-batch consistency evaluation of the site-directed coupling test strips at different hCG concentrations.

hCG/(mIU/mL)	T/C	Average of T/C	SD	CV (%)
2.5	0.336	0.339	0.004	1.05
	0.338			
	0.343			
	0.384			
10	0.392	0.386	0.006	1.63
	0.380			
	0.471			
	0.469			
20	0.478	0.473	0.005	1.63

Inter-batch consistency evaluation: Test strips from different batches were used to detect hCG at concentrations of 2.5, 10, and 20 mIU/mL. The corresponding T/C values were measured, and the coefficient of variation (CV) was calculated.

**Table S4.** Inter-batch consistency evaluation of the site-directed coupling test strips at different hCG concentrations.

hCG (mIU/mL)	T/C	Average of T/C	SD	CV (%)
2.5	0.327	0.354	0.029	8.09
	0.384			
	0.350			
10	0.334	0.401	0.058	14.54
	0.431			
	0.439			
20	0.510	0.543	0.047	8.70
	0.597			
	0.521			

## Data Availability Statement

The data supporting the results of this study are available within the article.