

## Supplementary Material

### **An indirect competitive assay-based method for the sensitive determination of tetracycline residue using a real-time fluorescence-based quantitative polymerase chain reaction**

Tianying Sheng, Hanyu Chen\*, Yingying, Lei, Baozhong Zhang, Huina Zhu

School of Environmental Engineering, Henan University of Technology, Zhengzhou 450001, PR China.

\* Corresponding author.

Telephone: +86 18623716700;

Fax: +86-0371-67756718;

E-mail: chy071@haut.edu.cn (H.Y. Chen);

Present address: School of Environment Engineering, Henan University of Technology, 100 Lianhua Road, Zhengzhou 450001, PR China.

**Table S1** Design of experiments for response surface methodology.

Factor	Name	Units	Low	High
A	Apt40	nM	100	125
B	sDNA	nM	100	125
C	cDNA	nM	75	100

**Table S2** Variance analysis for the established regression model.

Source	Sum of Squares	df <sup>a</sup>	Mean Square	F Value	P-value Prob > F	Significance <sup>b</sup>
Model	2.52	9	0.28	86.60	<0.0001	**
A	0.021	1	0.021	6.51	0.0381	*
B	0.25	1	0.25	76.96	<0.0001	**
C	0.11	1	0.11	34.20	0.0006	**
AB	0.71	1	0.71	218.50	<0.0001	**
AC	0.0056	1	0.0056	1.74	0.2284	
BC	0.11	1	0.11	32.71	0.0007	**
A <sup>2</sup>	0.71	1	0.71	220.79	<0.0001	**
B <sup>2</sup>	0.45	1	0.45	138.99	<0.0001	**
C <sup>2</sup>	0.050	1	0.050	15.49	0.0056	**
Residual	0.023	7	0.0032			
Lack of Fit	0.017	3	0.0057	4.17	0.1008	
Pure Error	0.0055	4	0.0014			
Cor Total	2.54	16				
R <sup>2</sup>					0.9911	
R <sup>2</sup> <sub>Adj</sub>					0.9797	
R <sup>2</sup> <sub>pred</sub>					0.8887	

<sup>a</sup> df: degree of freedom.

<sup>b</sup> \*\*: highly significant; \*: significant.

**Table S3** Comparison of several previously reported methods for tetracycline detection and the method developed in the present study.

Label	Analytical technique	LOD <sup>a</sup> (ng/mL)	Linear range (ng/mL)	Ref.
G-quadruplex	Colorimetric	1.47	-	1
Upconversion nanosensor	Fluorescence sensor	0.17	0.5-10 <sup>3</sup>	2
Polydimethylsiloxane plasma cavity	Surface-enhanced Raman scattering	0.28	15-55	3
Au NPs@MoS <sub>2</sub> /Ch	Electrochemical	13.33	2.22 × 10 <sup>-3</sup> -0.49	4
Aptamer-based competition	Fluorescent	7.02 × 10 <sup>-5</sup>	10 <sup>-3</sup> -10 <sup>3</sup>	Present study

<sup>a</sup> LOD: the limit of detection.

## References

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