

Supporting Information for

**A novel alkaline phosphatase assay based on the specific
chromogenic interaction between Fe³⁺ and ascorbic acid 2-
phosphate**

Linjie Wang,^a Kun Ye,^a Jianming Pan,^{a,*} Hongwei Song,^b Xiangheng Niu^{a,*}

^a Institute of Green Chemistry and Chemical Technology, School of Chemistry and
Chemical Engineering, Jiangsu University, Zhenjiang 212013, China

^b School of Environmental and Chemical Engineering, Jiangsu University of Science
and Technology, Zhenjiang 212003, China

* Corresponding author. E-mail: pjm@ujs.edu.cn; niuxiangheng@126.com

Experimental section

1. Chemicals and reagents

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, acetic acid (HAc), sodium acetate (NaAc), Na_3PO_4 , albumin, glutathione (GSH), threonine (Thr), casein, lysine (Lys), cysteine (Cys), and aspartic acid (Asp) were purchased from Sinopharm Chemical Reagent Co., Ltd. Ascorbic acid (AA) and alkaline phosphatase (ALP, 30 U mg^{-1} , from *Escherichia coli*) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. Ascorbic acid 2-phosphate (AAP) was provided by J&K Scientific Co., Ltd. Human serum samples were obtained from the Affiliated Hospital of Jiangsu University. Unless otherwise stated, all the chemicals and reagents were directly used without further purification. Deionized water was utilized throughout the study.

2. Chromogenic reaction of Fe^{3+} and AAP

Typically, 40 μL of 100 mM Fe^{3+} solution was first added to 2,910 μL of 0.1 M NaAc/HAc buffer (pH 3), and then 50 μL of 20 mM AAP solution was added to the buffer. After reaction for 60 s, the mixture was monitored by a Cary 8454 UV-Vis spectrometer (Agilent Technologies Co., Ltd.).

3. ALP detection based on the chromogenic reaction of Fe^{3+} and AAP

Typically, 50 μL of AAP (20 mM) and 200 μL of ALP (its activity varies, dissolved in Tris-HCl buffer, pH 8.8) were first incubated at 37°C for 2 h, and then 2,710 μL of 0.1 M NaAc/HAc buffer (pH 3) and 40 μL of 100 mM Fe^{3+} solution were added. After reaction for 60 s, the mixture was monitored by the spectrometer.

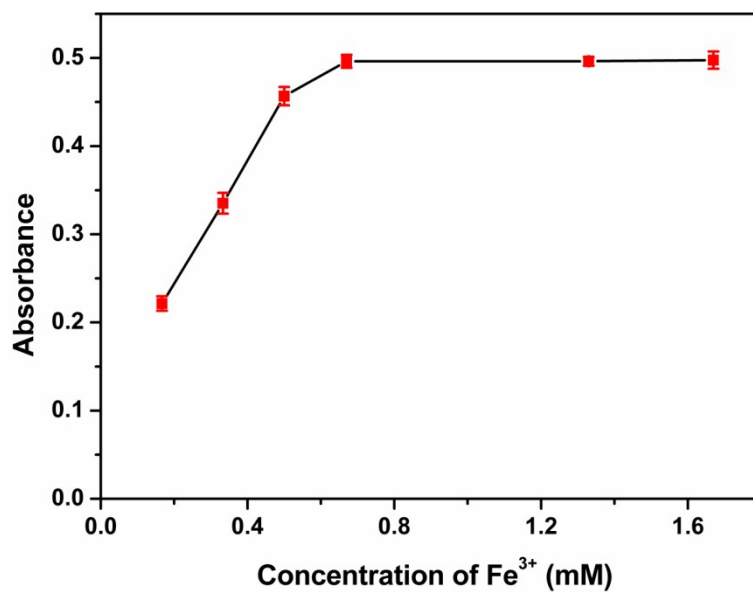


Figure S1. Effect of the Fe³⁺ concentration on the chromogenic reaction (AAP concentration: 0.33 mM).

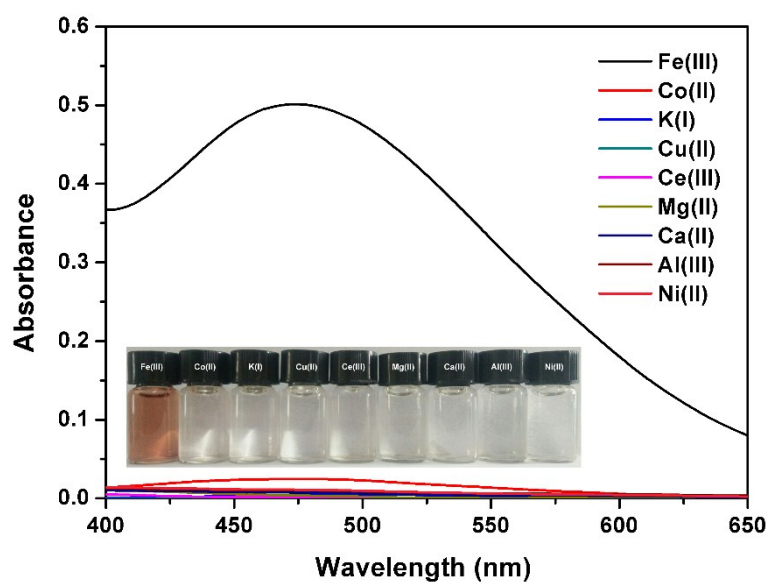


Figure S2. UV-Vis spectra of the possible reactions of various metal ions with AAP.

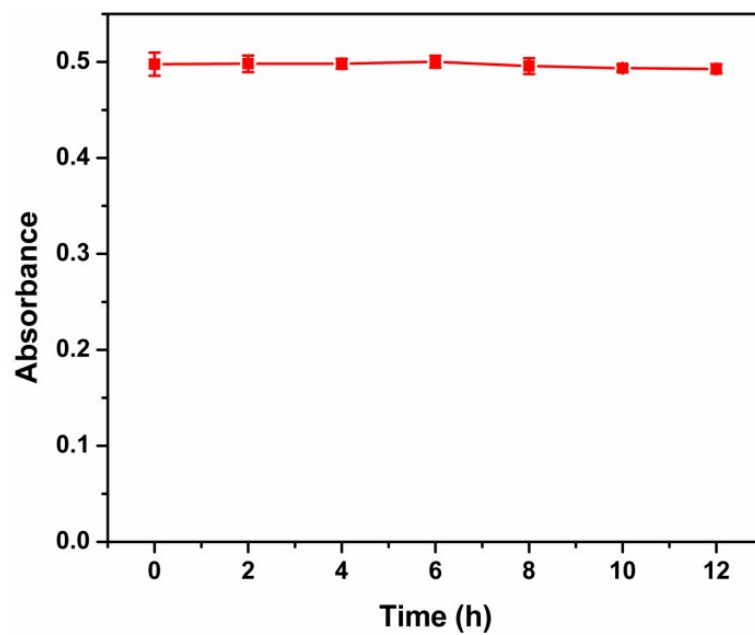


Figure S3. Stability of the Fe³⁺-AAP complex over time.

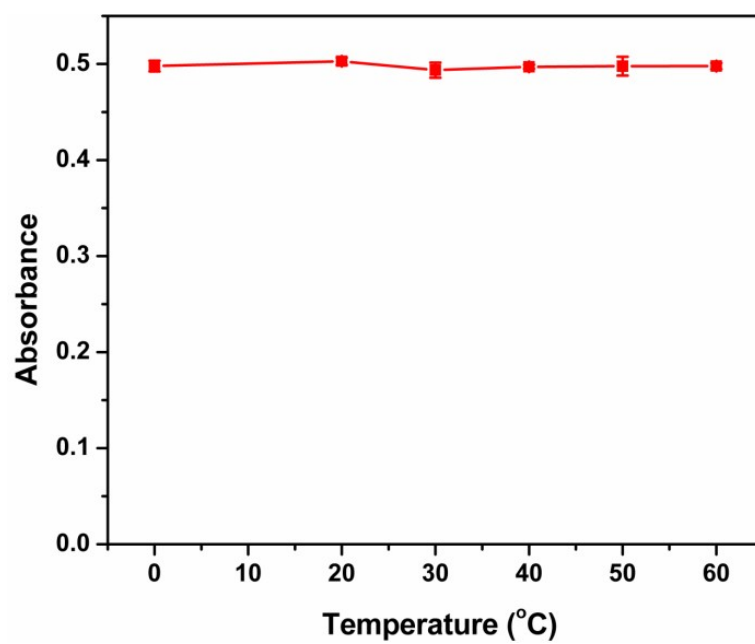


Figure S4. Stability of the Fe³⁺-AAP complex over temperature.

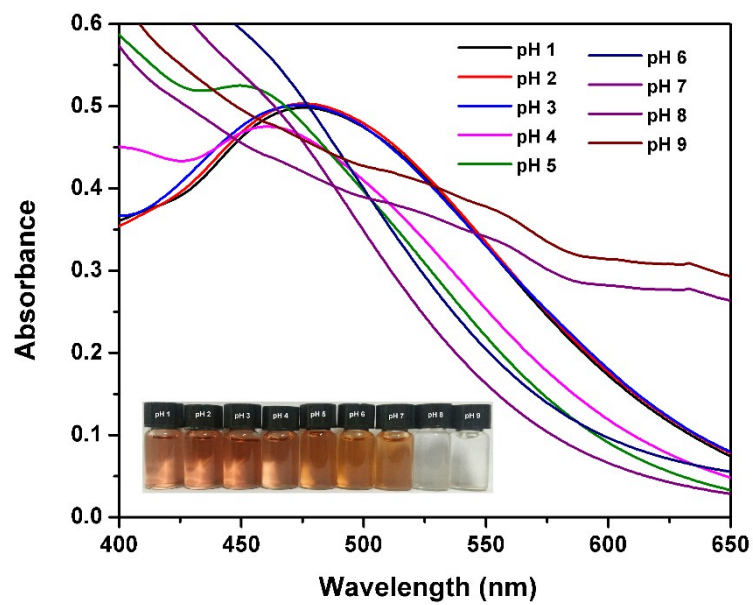


Figure S5. UV-Vis spectra of the mixture of Fe³⁺ and AAP in buffers with different pH values.

Table S1. Performance comparison of our ALP assay with other detection methods.

Principle	Method	Linear range (U L ⁻¹)	LOD (U L ⁻¹)	Ref.
ALP-triggered fluorogenic reaction of <i>o</i> -phenylenediamine and AA	Fluorometric	0.1~30	0.06	1
Pi-quenched oxidase-mimicking activity of Ce ⁴⁺	Colorimetric	0~50 50~250	2.3	2
ALP-triggered generation of a thiol quenching the fluorescence of silver nanoclusters	Fluorometric	0.08~2	0.02	3
AA-induced aggregation of fluorescent carbon dots	Fluorometric	0.2~6	0.16	4
AA-induced destroying of oxidase-like CoOOH nanoflakes	Colorimetric Fluorometric	0.04~160 0.04~160	0.032 0.026	5
Inhibition of fluorescent copper nanoparticles by PPI	Fluorometric	0.3~7.5	0.3	6
ATP hydrolysis-triggered dissociation of fluorescent cerium coordination polymer nanoparticles	Fluorometric	0.1~10	0.09	7
ALP-stimulated fluorescent coordination polymer nanoparticles	Fluorometric	25~200	10	8
Specific chromogenic reaction of Fe ³⁺ and AAP	Colorimetric	0.8~80	0.66	This work

References

1. D. Zhao, J. Li, C. Y. Peng, S. Y. Zhu, J. Sun and X. R. Yang, *Anal. Chem.*, 2019, **91**, 2978-2984.
2. H. W. Song, H. Y. Wang, X. Li, Y. X. Peng, J. M. Pan and X. H. Niu, *Anal. Chim. Acta*, 2018, **1044**, 154-161.
3. M. C. Luo, Z. Su, X. Y. Wang, L. Li, Y. F. Tu and J. L. Yan, *Microchim. Acta*, 2019, **186**, 180.
4. P. J. Ni, J. F. Xie, C. X. Chen, Y. Y. Jiang, Y. Z. Lu and X. Hu, *Microchim.*

- Acta*, 2019, **186**, 202.
5. S. G. Liu, L. Han, N. Li, N. Xiao, Y. J. Ju, N. B. Li and H. Q. Luo, *J. Mater. Chem. B*, 2018, **6**, 2843-2850.
 6. L. L. Zhang, J. J. Zhao, M. Duan, H. Zhang, J. H. Jiang and R. Q. Yu, *Anal. Chem.*, 2013, **85**, 3797-3801.
 7. C. X. Chen, Q. Yuan, P. J. Ni, Y. Y. Jiang, Z. L. Zhao and Y. Z. Lu, *Analyst*, 2018, **143**, 3821-3828.
 8. J. J. Deng, P. Yu, Y. X. Wang and L. Q. Mao, *Anal. Chem.*, 2015, **87**, 3080-3086.