

Electronic Supporting Information (ESI)

**Spectrophotometric determination of ethyl carbamate  
through bi-enzymatic cascade reactions**

Xiaoxia Lu, Nandi Zhou and Yaping Tian\*

*The Key Laboratory of Industrial Biotechnology, Ministry of Education, School of  
Biotechnology, Jiangnan University, Wuxi 214122, China. Fax: +86 510 85918116;  
Tel: +86 510 85918116; E-mail: [biochem@jiangnan.edu.cn](mailto:biochem@jiangnan.edu.cn)*

## Experimental sections

### *Materials and reagents*

$\beta$ -nicotinamide adenine dinucleotide, reduced disodium salt hydrate (NADH) was purchased from Sangon Biotech Co., Ltd.  $\alpha$ -ketoglutarate was obtained from Fluka. Ethyl carbamate was procured from Aladdin Chemistry Co., Ltd. Glutamate dehydrogenase (GLDH, from bovine liver) was purchased from Nanjing Duly Biotech Co., Ltd. Urethanase was produced from strain *Penicillium variable* JN-A525 which was screened by our laboratory.<sup>S1</sup> The purified urethanase was prepared through our previously reported methods.<sup>S2</sup> All other chemicals were of analytical grade. Ultrapure water (18.2 M $\Omega$  cm) was employed to prepare all aqueous solutions.

Stock solutions of  $\alpha$ -ketoglutarate and NADH were freshly prepared using ultrapure water. To investigate the effect of pH on the cascade reactions, urethanase (56 units/mL) and GLDH (152 units/mL) were prepared with 25 mM citrate buffer (pH4.5-5.5), phosphate buffer (pH6.0-7.5) and Tris-HCL buffer (pH7.5-8.0), respectively.

### *UV-visible spectrophotometric determination*

All absorption measurements were conducted by using a UV-visible spectrophotometer (UV 600PC spectrophotometer, MAPADA, China). The volume of reaction solution was fixed at 600  $\mu$ L in 25 mM phosphate buffer (pH 6.0) containing certain concentration EC,  $\alpha$ -ketoglutarate, NADH, urethanase and GLDH. The absorbance at 340 nm was monitored for 5 min. The change of absorbance ( $\Delta A_{340}$ ) before and after 5 min reaction was calculated and used to quantitatively analyze the concentration of EC.

$$\Delta A_{340} = A_{340 \text{ before}} - A_{340 \text{ after}}$$

## References

S1 N.-d. Zhou, X.-l. Gu, Y.-p. Tian, *Appl. Biochem. Biotechnol.*, 2013, **170**, 718.

S2 N.-d. Zhou, X.-l. Gu, X.-h. Zha, Y.-p. Tian, *Appl. Biochem. Biotechnol.*, 2014, **172**,

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