

## *Electronic Supplementary Information*

# Design and functionalization of a μPAD for enzymatic determination of nitrate ions in urine

Francisca T. S. M. Ferreira, Raquel B. R. Mesquita, António O. S. S. Rangel

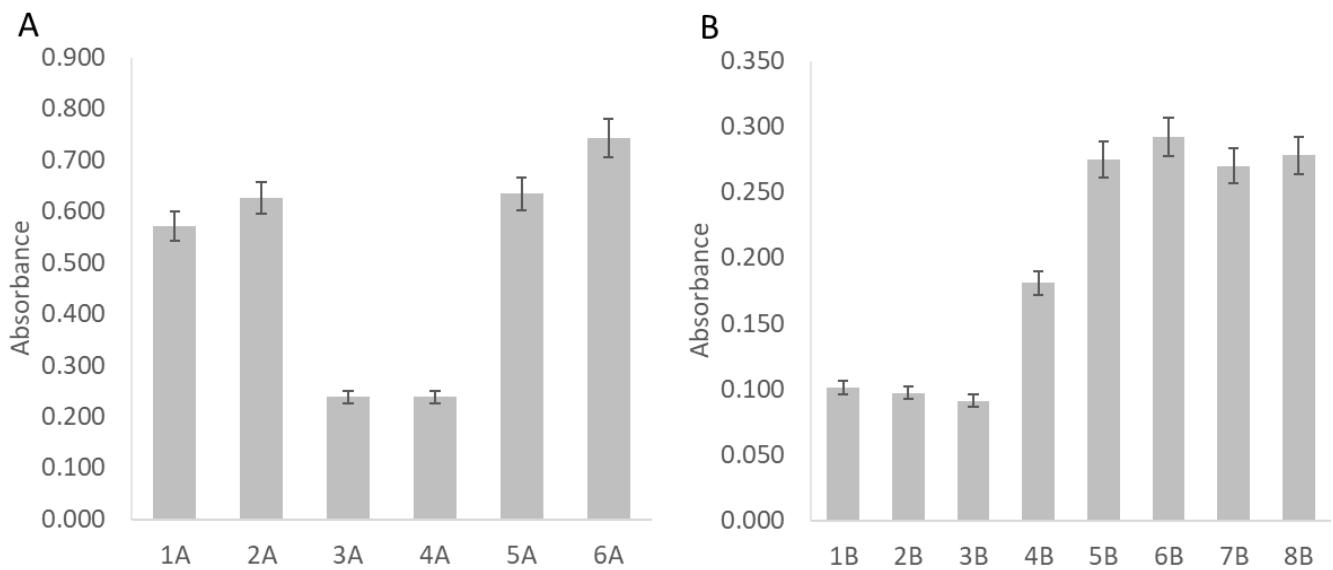
*Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005, Porto, Portugal*

**Table S1.** Description of the different parameters tested under each condition and the resulting absorbance signal: 100 µL of a nitrate standard of 12 mM (1.2 µmol); concentration of NADPH, 2 mM; concentration of NR, 0.4 U/mL.

Condition ID	NADPH		NR		A
	Vol. (µL)	µmol	Vol. (µL)	U	
1A	100	0.2	100	0.04	0.572
2A	50	0.1	100	0.04	0.626
3A	100	0.2	50	0.02	0.238
4A	50	0.1	50	0.02	0.238
5A	50	0.1	150	0.06	0.634
6A	50	0.1	200	0.08	0.742

**Table S2.** Description of the different parameters tested under each condition and the resulting absorbance signal: 100 µL of a nitrate standard of 1.2 mM (0.12 µmol); concentration of NADPH, 0.2 mM; concentration of NR, 0.4 U/mL.

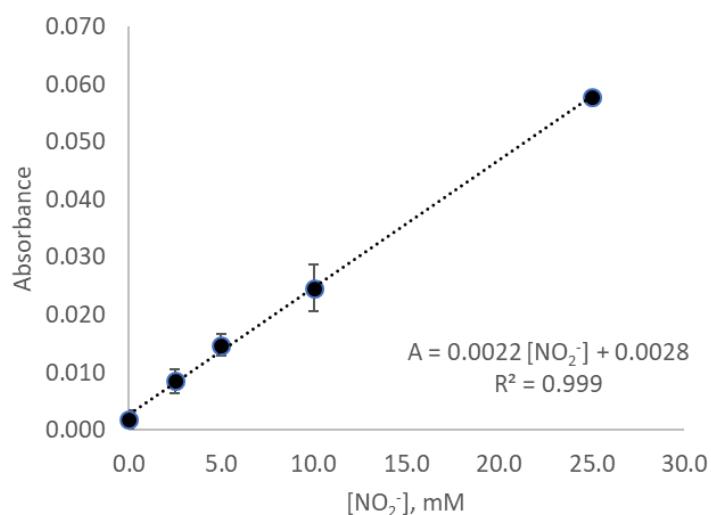
Condition ID	NADPH		NR		A
	Vol. (µL)	µmol	Vol. (µL)	U	
1B	50	0.01	100	0.04	0.101
2B	50	0.01	150	0.06	0.097
3B	50	0.01	200	0.08	0.091
4B	100	0.02	100	0.04	0.181
5B	150	0.03	100	0.04	0.275
6B	200	0.04	100	0.04	0.292
7B	150	0.03	150	0.06	0.270
8B	150	0.03	200	0.08	0.278



**Figure S1.** Plot of the studied conditions in ESI Table1 and Table S2 with: A) a 12 mM nitrate standard; and B) a 1.2 mM nitrate standard; the error bars represent 5% relative deviation.

**Table S3.** Summary of some properties of Whatman Filter Papers used

<i>Treatment</i>	<i>Paper</i>	<i>Particle Retention (<math>\mu\text{m}</math>)</i>
<i>Qualitative</i>	1	11
	4	20-25
<i>Hardened Ashless</i>	541	20-25



**Figure S2.** Nitrite calibration curve; the error bars represent the standard deviation.