

Supplementary files

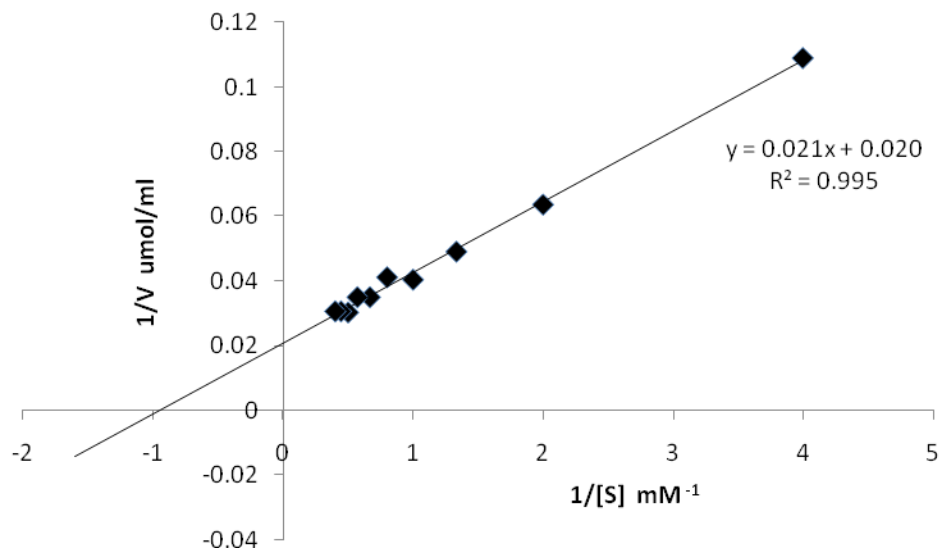


Figure 1 .Lineweaver- Burk plot for Lpc53E1 activity with *p*- nitrophenyl palmitate as substrate.

Kinetics study by Lineweaver-Burk approach

The kinetics of Lpc53E1 were determined by performing a Lineweaver- Burk plot with with different initial *p*- nitrophenyl palmitate concentrations (0.25- 2.5 mM). Reactions were initiated by the addition of 10nM of lipase in Tris buffer pH 8.2 and incubated at 40 °C. The V_{max} and K_M values were determined by linear regression using the following Lineweaver- Burk equation:

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max} [S]} = \frac{K_m}{V_{max} [S]} + \frac{[S]}{V_{max} [S]} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

The K_M and V_{max} value of Lipase was calculated from the slope (K_M/V_{max}) and the intercept ($1/V_{max}$) of Lineweaver –Burk plot.

Table 1. Experiments design matrix of response surface methodology (CCD) with experimental and predicted values of the lpc53E1 activity.

<i>Run</i>	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>Relative activity(%)</i>	
					<i>X₁</i>	<i>X₂</i>
1	-1.000	-1.000	-1.000	-1.000	76.72	73.17
2	1.000	-1.000	-1.000	-1.0	70.21	70.96
3	-1.000	1.000	-1.000	-1.000	78.25	78.95
4	1.000	1.000	-1.000	-1.000	58.23	58.46
5	-1.000	-1.000	1.000	-1.000	45.73	47.56
6	1.000	-1.000	1.000	-1.000	62.17	64.51
7	-1.000	1.000	1.000	-1.000	60.12	59.68
8	1.000	1.000	1.000	-1.000	58.21	58.36
9	-1.000	-1.000	-1.000	1.000	52.43	50.46
10	1.000	-1.000	-1.000	1.000	53.77	57.61
11	-1.000	1.000	-1.000	1.000	67.89	68.94
12	1.000	1.000	-1.000	1.000	61.47	57.82
13	-1.000	-1.000	1.000	1.000	53.67	56.84
14	1.000	-1.000	1.000	1.000	85.67	83.14
15	-1.000	1.000	1.000	1.000	84.24	81.67
16	1.000	1.000	1.000	1.000	82.76	89.71
17	-2.000	0.000	0.000	0.000	52.78	54.46
18	2.000	0.000	0.000	0.000	63.54	60.29
19	0.000	-2.000	0.000	0.000	53.86	52.71
20	0.000	2.000	0.000	0.000	65.46	65.04
21	0.000	0.000	-2.000	0.000	76.43	78.53
22	0.000	0.000	2.000	0.000	88.48	84.81
23	0.000	0.000	0.000	-2.000	62.45	62.23
24	0.000	0.000	0.000	2.000	72.23	70.87
25	0.000	0.000	0.000	0.000	100	100
26	0.000	0.000	0.000	0.000	100	100
27	0.000	0.000	0.000	0.000	100	100
28	0.000	0.000	0.000	0.000	100	100
29	0.000	0.000	0.000	0.000	100	100
30	0.000	0.000	0.000	0.000	100	100

A: *p*NPP (mM): -1(0.5), 0 (1.0), +1(1.5) , B: Ca⁺(mM): -1(0), 0 (5), +1(10) C: Reaction time (Min): -1 (5), 0 (15), +1 (25) and D: NaCl (M) : -1 (4), 0(5), +1(6).

<i>Source</i>	<i>Sum of</i>	<i>df</i>	<i>Mean</i>	<i>F Value</i>	<i>p-value</i>
	<i>Squares</i>		<i>Square</i>		<i>Prob>F</i>
Model	8717.35	14	622.67	57.73	< 0.0001***
<i>A-pNPP</i>	50.93	1	50.93	4.72	0.0462**
<i>B-Ca+</i>	228.17	1	228.17	21.15	0.0003**
<i>C-Reaction time</i>	59.22	1	59.22	5.49	0.0333**
<i>D-NaCl</i>	111.89	1	111.89	10.37	0.0057**
<i>AB</i>	333.98	1	333.98	30.96	< 0.0001***
<i>AC</i>	367.30	1	367.30	34.05	< 0.0001***
<i>AD</i>	87.61	1	87.61	8.12	0.0122***
<i>BC</i>	40.26	1	40.26	3.73	0.0725***
<i>BD</i>	161.54	1	161.54	14.98	0.0015***
<i>CD</i>	1023.36	1	1023.36	94.88	< 0.0001***
<i>A²3</i>	115.16	1	3115.16	288.82	< 0.0001***
<i>B²</i>	2899.78	1	2899.78	268.86	< 0.0001***
<i>C²</i>	576.19	1	576.19	53.42	< 0.0001***
<i>D²</i>	1917.93	1	1917.93	177.82	< 0.0001***
Residual	161.78	15	10.79		
<i>Lack of Fit</i>	161.78	10	16.18		
<i>Pure Error</i>	0.000	5	0.000		
Cor Total	8879.14	29			

Table 2. Regression analysis of enzyme assay conditions of lpc53E1 for quadratic response surface model fitting (ANOVA)

*** More significant ** significant level with $P < 0.05$