

**Table 1.** Apparent Kinetic Constants for Selected CALB Variants with *p*-Nitrophenol butyrate and DiFMU Octanoate as Substrates

enzyme variants		<i>p</i> -nitrophenol butyrate				DiFMU octanoate			
name <sup>a</sup>	sequence <sup>b</sup>	$K_M$ ( $\mu$ M)	$k_{cat}$ ( $\text{min}^{-1}$ )	$k_{cat}/K_M$ ( $\text{min}^{-1} \mu\text{M}^{-1}$ )	relative specificity <sup>c</sup>	$K_M$ ( $\mu$ M)	$k_{cat}$ ( $\text{min}^{-1}$ )	$k_{cat}/K_M$ ( $\text{min}^{-1} \mu\text{M}^{-1}$ )	relative specificity <sup>c</sup>
wild type	L1/P317	410 $\pm$ 40	305 $\pm$ 10	0.74	1.0	2.6 $\pm$ 0.3	2 $\pm$ 0.1	0.8	1.0
cp44	G44/T43	690 $\pm$ 90	6 $\pm$ 0.5	0.01	0.01	5.6 $\pm$ 0.8	0.5 $\pm$ 0.05	0.1	0.13
cp144	L144/A141	550 $\pm$ 50	178 $\pm$ 7	0.32	0.4	2.0 $\pm$ 0.5	1 $\pm$ 0.1	0.5	0.6
cp148	A148/L147	500 $\pm$ 30	171 $\pm$ 4	0.34	0.5	3.5 $\pm$ 0.5	1.5 $\pm$ 0.2	0.35	0.4
cp150	S150/V149	510 $\pm$ 90	520 $\pm$ 45	1.02	1.4	2.7 $\pm$ 0.8	2.1 $\pm$ 0.2	0.8	1.0
cp283	A283/A283-KRPRINSP	280 $\pm$ 50	2971 $\pm$ 180	10.61	14.3	2.5 $\pm$ 0.5	25 $\pm$ 1.4	10.9	13.6
cp284	A284/A287-KRPRINSP	550 $\pm$ 70	2980 $\pm$ 200	5.42	7.3	8.8 $\pm$ 1.0	34 $\pm$ 4	3.8	4.8
cp289	P289/A284-KRPRINSP	260 $\pm$ 30	3258 $\pm$ 215	12.53	16.9	5.5 $\pm$ 1.0	120 $\pm$ 7	23	28.8
cp294	E294/A283	310 $\pm$ 40	73 $\pm$ 4	0.23	0.3	9.5 $\pm$ 2.0	2.6 $\pm$ 0.34	0.3	0.4

<sup>a</sup> CALB nomenclature: cp44 = circularly permuted protein whose N-terminus starts at amino acid 44 of the wild-type sequence. <sup>b</sup> N- and C-terminal amino acids (all in single-letter code) are listed. Small variations in chain length of individual permutants are caused by reading frame shifts and staggered ends upon DNaseI digestion. <sup>c</sup> Relative specificity =  $k_{cat}/K_M$  (variant)/ $k_{cat}/K_M$  (wild type).