

Table 3: Values of the Steady-State Parameters  $k_{\text{cat}}$  and  $K_M$  for *NaeI* Endonuclease with Different Substrates

substrate	$k_{\text{cat}}$ ( $\text{s}^{-1}$ )	$K_M$ (nM)	$k_{\text{cat}}/K_M$ ( $\text{M}^{-1} \text{s}^{-1}$ )	$k_{\text{st}}$ ( $\text{s}^{-1}$ )
M13mp18 <sup>a</sup>	$(4.5 \pm 0.5) \times 10^{-2}$	$10 \pm 3$	$(5 \pm 2) \times 10^6$	$\geq 0.2$
M13-14 <sup>a</sup>	$(4 \pm 0.4) \times 10^{-1}$	$(1.7 \pm 0.2) \times 10^2$	$(2.4 \pm 0.5) \times 10^6$	nd
M13-14 nc <sup>b</sup>	$(3 \pm 1) \times 10^{-7}$ <sup>c</sup>	$(1-3) \times 10^4$	$(7-40) \times 10^{-3}$	nd

<sup>a</sup> Reaction conditions used are as described under Materials and Methods, with 0.21 nM *NaeI* and 100 nM DNA fragment HA. <sup>b</sup> With 100 nM *NaeI*. <sup>c</sup> Determined from velocities of cleavage reactions performed at 100 and 200  $\mu\text{M}$  substrate. All values listed are determined from at least three independent experiments.