

Table 3  
6PF2K and Fru-2,6-P<sub>2</sub>ase activity of HBP wild type and mutants

Enzyme	6PF2K			Fru-2,6-P <sub>2</sub> ase
	$K_M^{F6P}$ ( $\mu$ M)	$k_{cat}$ ( $10^{-3}$ s <sup>-1</sup> )	$K_i^{F6P}$ ( $\mu$ M)	$k_{cat}$ ( $10^{-3}$ s <sup>-1</sup> )
HBP1	7.1 ± 0.8	407 ± 22	9900 ± 400	0.113 ± 0.011
HBP1 N-6His	8.0 ± 0.9	430 ± 22	12200 ± 2200	0.062 ± 0.011
HBP1 His-253 > Ala	7.3 ± 0.1	391 ± 2	11100 ± 2200	0.222 ± 0.009
HBP1 N-6His/Ser-302 > Arg	9.5 ± 0.6	399 ± 40	10900 ± 1200	66.2 ± 4.2
HBP1 Ser-460 > Asp	6.7 ± 0.4	394 ± 22	9200 ± 300	0.078 ± 0.008
HBP1 Thr-470 > Asp	6.6 ± 0.2	435 ± 37	13000 ± 1800	0.077 ± 0.009
HBP1 Ser-477 > Asp	6.4 ± 0.9	405 ± 70	9700 ± 300	0.078 ± 0.008
HBP1 His-253 > Ala/Ser-460 > Asp	7.1 ± 0.0	401 ± 16	9500 ± 600	0.192 ± 0.010
HBP1 Ser-460 > Asp/Thr-470 > Asp	6.6 ± 0.0	394 ± 18	8500 ± 800	0.085 ± 0.005
HBP1 N-6His/ $\Delta$ C	9.2 ± 1.0	696 ± 54	9600 ± 1000	ND
HBP2 N-6His	7.4 ± 0.0	447 ± 32	11800 ± 100	0.477 ± 0.035

The 6PF2K activity was measured at pH 7.5 with 5 mM ATP and 1–5000  $\mu$ M Fru-6-P. The Fru-2,6-P<sub>2</sub>ase activity was measured at pH 7.5 with 50  $\mu$ M Fru-2,6-P<sub>2</sub>.

ND—not determined.