

Supplementary Material

Figure. 1 Physical properties of At5g36160 effects of temperature and pH on enzyme activity. The transamination reaction was carried out using the forward assay using L-Tyrosine (10 mM) as the amino donor and 2-ketoglutarate (10 mM) as the amino acceptor by measuring the production of 4-hydroxyphenylpyruvate at 320 nm.

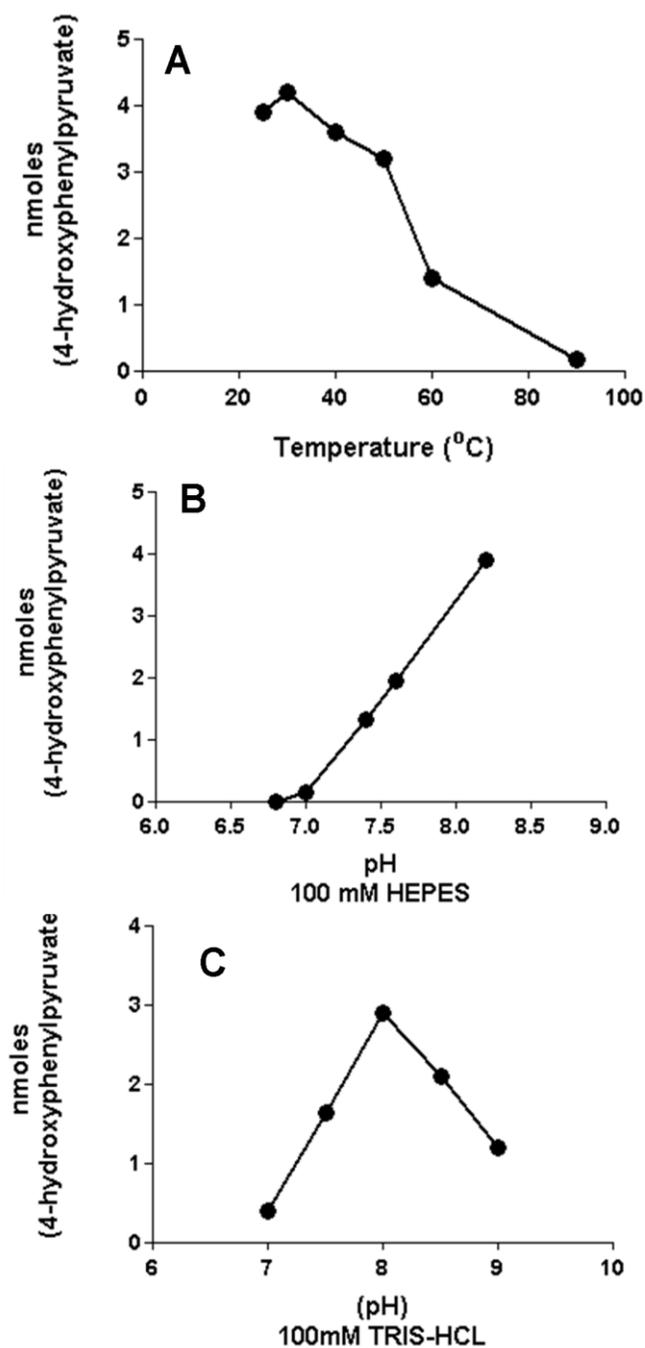
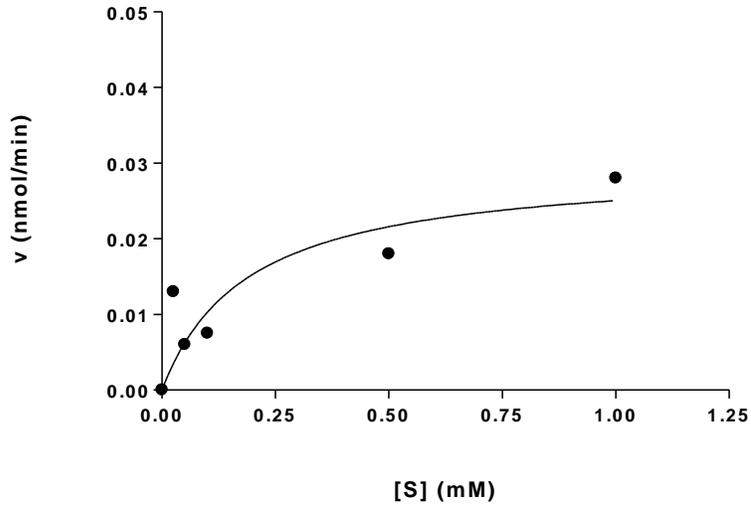
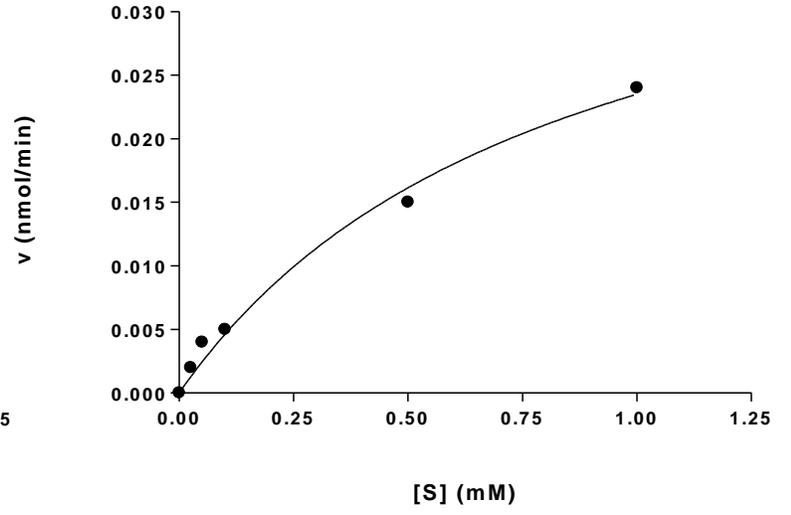


Figure. 2

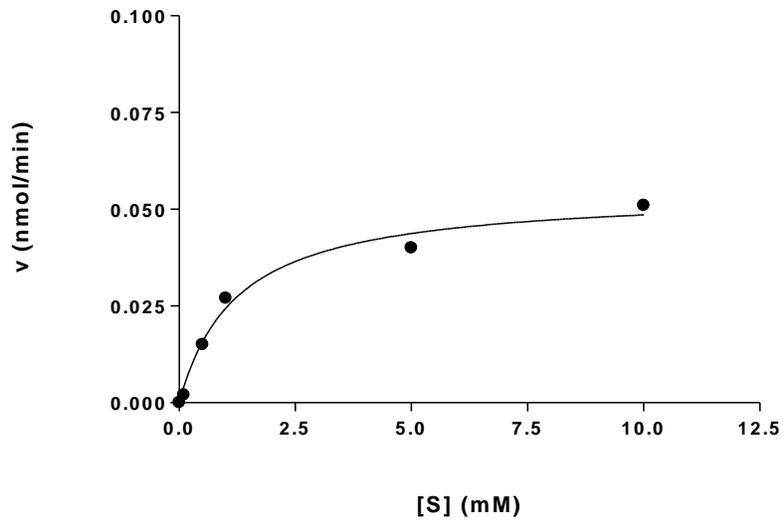
At5g36160 K_m Tyrosine



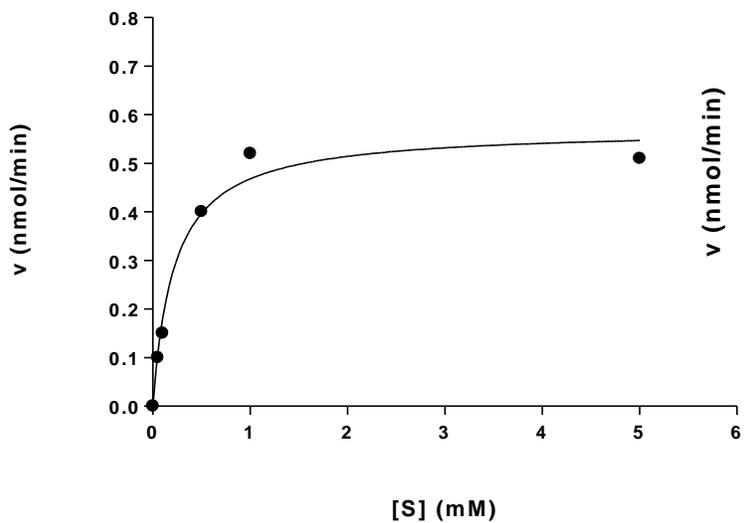
At5g36160 K_m Phenylalanine



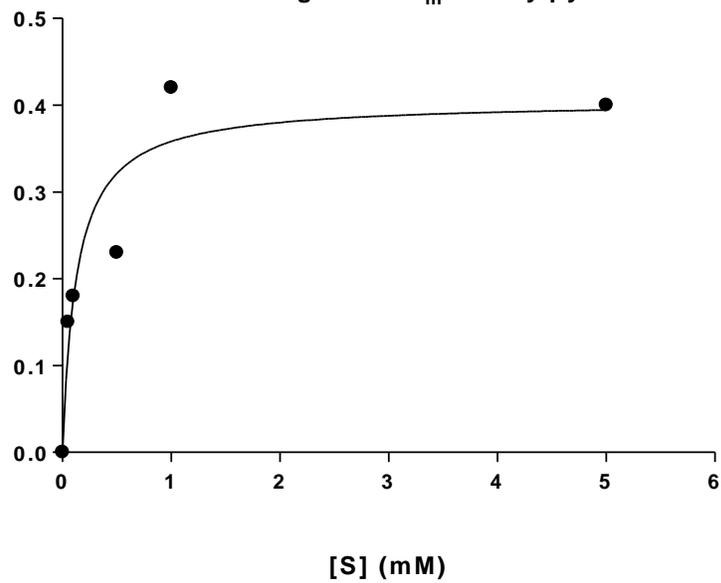
At5g36160 K_m 2-ketoglutarate



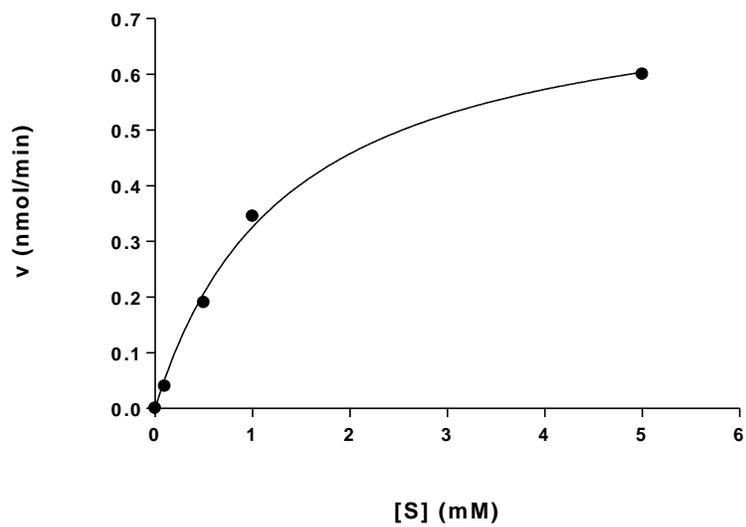
**At5g36160 K_m
4-hydroxyphenylpyruvate**



At5g36160 K_m Phenylpyruvate



At5g36160 K_m Glutamate



Michaelis-Menten plots of At5g36160. The enzyme assays used to draw the Michaelis-Menten plots are described in the “materials and methods” section. V_{max} is represented in nmoles/min, the K_m of the various substrates is represented in millimolar (mM). The measurements were made under optimal conditions with respect to pH, temperature and substrate concentrations. The kinetic constants were calculated by non-linear regression analysis using the GraphPad Prism Version 3.03 software.

2-ketoglutarate 10µg recombinant enzyme
 5 mM L-Phenylalanine

L-Tyrosine 10µg recombinant enzyme
 20mM 2-ketoglutarate

L-Phenylalanine 10µg recombinant enzyme
 20mM 2-ketoglutarate

4-hydroxyphenylpyruvate 20µg recombinant enzyme
 0.3mM thio-NAD
 0.3mM Coenzyme A
 5mM L-Glutamate
 200µg recombinant 2-ketoglutarate dehydrogenase

Phenylpyruvate 20µg recombinant enzyme
 0.3mM thio-NAD
 0.3mM Coenzyme A
 5mM L-Glutamate
 200µg recombinant 2-ketoglutarate dehydrogenase

L-Glutamate 20µg recombinant enzyme
 0.3mM thio-NAD
 0.3mM Coenzyme A
 5mM 4-hydroxyphenylpyruvate
 200µg recombinant 2-ketoglutarate dehydrogenase

Note: the assays were in 500µl final volume using 100mM HEPES pH (8.2)