Assay of β-Glucosidase

Laxman Manjhi 2019BB10034

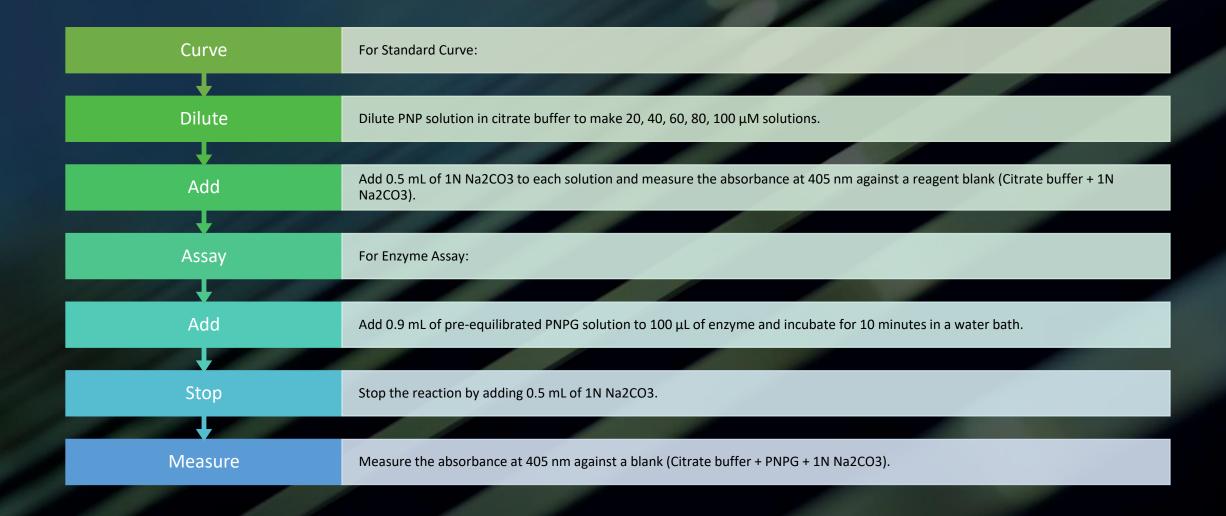
Ratnesh Kumar Sharma 2019BB10047



Background

- β-Glucosidase is an enzyme that breaks down β 124 bonds in glucose or glucose-substituted molecules
- It releases glucose from β-D-glucosides
- Activity can be measured by hydrolysis of p-nitrophenol-β-D-glucopyranoside (PNPG)
- PNP formed by hydrolysis is proportional to the amount of β -glucosidase at the time of reaction
- PNP absorbance can be measured at 405 nm
- Reaction is stopped by adding Na2CO3 which shifts the pH to alkaline (pH = 11.0)
- PNP is converted to yellow-colored anionic form and β -glucosidase is inactivated at alkaline pH.

Method

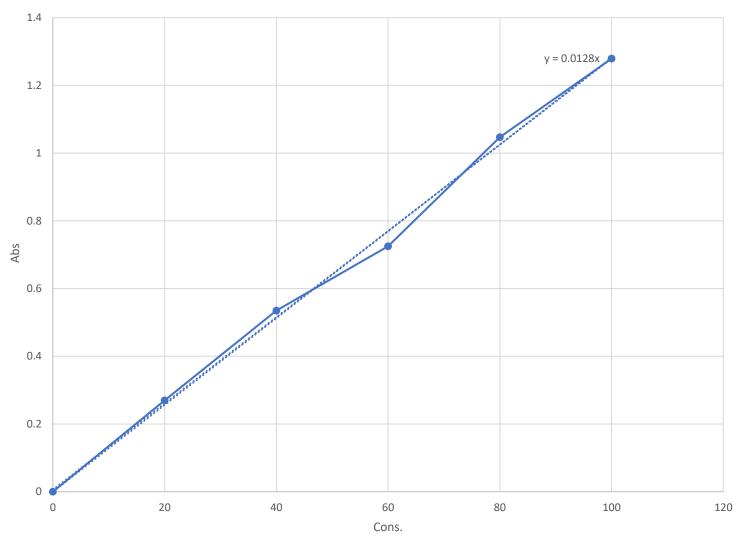


Observation

Conc µmol	Absorbance
0	0
20	0.27
40	0.535
60	0.725
80	1.047
100	1.279
Unknown	
50C	1.154
RT	0.443

Conc. vs Abs plot





Calculation

Conc. of PNP released at 50C = 90 µmol

Activity of enzyme = (90*10)/(10*0.1) = 900 IU/mL

Conc. of PNP released at RT = 34.60 µmol

Activity of enzyme = (34.60*10)/(10*0.1) = 346 IU/mL

