Saptasense Protocol

ChOx Enzyme Activity Testing

Background

We will be measuring ChOx activity using the Cayman Chemical H_2O_2 Kit, which produces a colorimetric readout in the presence of the H_2O_2 byproduct of the oxidation of choline. We can quantify the amount of product formed by measuring the absorbance of the resulting solution.

Materials

1X PBS
Detector ADHP
Horseradish Peroxidase
Enzyme of interest
Enzyme substrate
Pure water

Instruments

Quartz cuvette or NanoDrop
96 well plate
96 well plate reader

I. Prepare Solutions

Methods

- ☐ Dilute the H_2O_2 standard solution 1:1000 in pure water and read the absorbance at 240 nm using a quartz cuvette or a NanoDrop
  - ☐ Calculate the H_2O_2 concentration: $[H_2O_2] = \frac{absorbance \times dilution\ factor}{43.6}$
  - ☐ Prepare 1 mL of 1mM H_2O_2 in PBS
- ☐ Prepare HRP enzyme reaction solution **(Do this < 1 hour before starting the assay)**
  - ☐ For one 96 well plate, add 100 uL detector ADHP and 100 uL horseradish peroxidase to 800 mL PBS. Adjust based on how many wells you're running
- ☐ Label eight tubes A-H. Add the following amount of 1 mM H2O2 and PBS to each tube:

Tube	1 mM H_2O_2	Assay Buffer (uL)	Final Concentration (uM)
A	80	920	80
B	60	940	60
C	40	960	40
D	20	980	20
E	10	990	10
F	5	995	5
G	2.5	997.5	2.5
H	0	1000	0

II. ChOx Activity Assay

- ☐ Add 10 uL PSB to the sample activity wells
- ☐ Transfer the desired amount of enzyme substrate to the corresponding sample wells (will vary)
- ☐ Transfer 70 uL of each standard from tubes A-H to the standard wells of a 96 well plate
- ☐ Add 10 uL HRP enzyme reaction solution to each well
- ☐ Add the desired amount of enzyme to the corresponding sample wells (will vary)
- ☐ Read the absorbance at 570 nm every few seconds until the graph of absorbance versus time reaches a plateau

Volumes in each well type:

	Standard (uL)	PBS (uL)	HRP enzyme reaction sln (uL)	Enzyme substrate (uL)	Enzyme (uL)
Standard Wells	70	0	10	0	0
Sample Activity Wells	0	10	10	0-60 uL	0-60 uL

*The combined volume of enzyme and enzyme substrate added should not exceed 60 uL. If the combined volume is less than 60 uL, add enough assay buffer to the well to bring the total well volume to 80 uL

III. Enzyme Activity Calculations

- ☐ Construct the standard curve:
  - ☐ Calculate the average absorbance of each standard and sample
  - ☐ Subtract the average absorbance of the blank (Standard H) from itself and from all other standards and samples to get the corrected absorbances
  - ☐ Plot the corrected absorbance of each standard from step 2 as a function of the final H2O2 concentration (μM)
- ☐ Calculate the H_2O_2 concentration of the samples using the equation obtained from the linear regression of the standard curve:

$$\Delta H_2O_2\ (uM) = \frac{corrected\ absorbance - y\ intercept}{slope}$$

- ☐ Using the values obtained, construct a Michaelis-Menten curve

Protocol adapted from:
https://cdn.caymanchem.com/cdn/insert/600050.pdf