Tyrosinase-Lysate Characterization

Objective

Establish a time, concentration, temperature, and pH profile of tyrosinase activity in cell lysate.

Assay

After incubating the reactions with their set parameters, stop the reaction with H_3PO_4 or other reaction-stopping reagent.

The products of the reaction will then be dropped on a slide and a toothpick will be placed on the slide. The "stickiness" will be determined by whether or not the toothpick sticks. Other assays, like Coomassie-Blue staining may also be used to create a more quantitative analysis.

Materials

Tyrosinase Stock Solution (1 mg/mL in 7.4 pH Phosphate Buffer—0.05 M)
Remember to store **FROZEN**
Cell Lysate (the concentration of the lysate will be determined by a Bradford Assay)
Phosphate Buffer (to adjust pH)
Reaction stopper (current plan: 50% H_3PO_4 in H_2O)
Incubator
Glass slide
Toothpick

Time Profile

Stop incubation at the following times:
2 min, 4 min, 6 min, 8 min, 10 min, 15 min.

If no strange time profile is found, then proceed at 10 min incubation for the next profiles.

Temperature: 25 deg C
pH: 6.5 or 7.0
Tyrosine Concentration: determine after Bradford Assay.

Tyrosinase Concentration Profile

Our tyrosinase specs: 5370 units/mg

From stock of 1 mg/mL, test with 2.5 mL each:
-1 mg/mL (5370 units/mL)
-0.1 mg/mL (537 units/mL)
-0.01 mg/mL (53 units/mL)
-Negative Control: Phosphate buffer

Find optimum concentration and work with that value in the next set of experiments.

Temperature: 25 deg C
pH: 6.5 or 7.0

Temperature Profile

Test incubation temperatures at (all in deg C):
20 22 24 26 28
30 32 34 36 38
40 42 44 46 48
50

Find optimum temperature for the next set of experiments.

pH: 6.5 or 7.0

pH Profile

Test mixtures at following pHs, varying pH by adjusting the buffer solution:

4.0 4.2 4.4 4.6 4.8
5.0 5.2 5.4 5.6 5.8
6.0 6.2 6.4 6.6 6.8
7.0 7.2 7.4 7.6 7.8
8.0