Tyrosine Phenol Lyase
February 21, 2003

Summary

Tyrosine phenol lyase catalyzes the reversible conversion of tyrosine to phenol, pyruvate and ammonium. This protocol describes a coupled enzyme assay involving an excess of lactate dehydrogenase and NADH into the reaction mixture. Lactate dehydrogenase catalyzes the reversible reduction of pyruvate to lactate using NADH as a co-substrate. Thus, the principle of the assay is that pyruvate formed is quantitatively converted into lactate, allowing the measurement of the reduction in NADH.

Solutions Required

1. 0.50 M HEPES buffer pH = 8.0

2. 2.0 M KCl

3. 10 mM dithiothreitol (DTT)

4. 1.0 mM pyridoxal 5-phosphate (PLP)

5. 6.4 mM NADH
   must be prepared fresh

6. 10,000 U/mL lactate dehydrogenase (LDH)
   as purchase from Sigma (cat. L2500)

7. 10 mM tyrosine
   prepare by gentle warming in 20 mM HCl

Preparation of Cell Extract

Follow general protocol **Preparation of Cell Extract**.

1. After first pelletization of cells, resuspend at 4°C in potassium phosphate buffer.
2. After second pelletization of cells, resuspend at 4°C in potassium phosphate buffer.

Spectrophotometer

Turn on the ultraviolet bulb on the spectrophotometer (Beckman DU50) and wait 10 minutes for warm-up. Select the kinetics-time window on the instrument. Load the method "A:/nadh30" or "A:/nadh37". These methods each have a run-time of 60 s, a temperature of 30°C or 37°C (respectively), a wavelength of 340 nm and use 2 autosamplers.

Procedure

1. For each assay, prepare the two cocktails shown in the following table into two separate UV-translucent cuvettes, and keep them on ice.

	Volume (µL) added to:	
Solution	Control	Experimental
DI H_2O	460	210
HEPES	200	200
KCl	100	100
DTT	50	50
PLP	50	50
NADH	30	30
LDH	10	10
tyrosine	0	250

2. Directly from the ice when ready to commence the assay, place the two cuvettes (each containing 900 µL†) into the spectrophotometer holder (position #1 for control, position #2 for experimental). Use cuvette lid caps to mix 3 or 4 times then insert in instrument.
3. Wait 10 minutes to allow the temperature of the solutions in the cuvettes to equilibrate.
4. "Blank" and then depress "Read Samples" on the monitor.
5. Simultaneously add 100 µL† of the cell extract to the cuvettes.
6. To mix solutions, immediately and simultaneously aspirate and dispense the contents of the cuvettes with a pipettor.
7. Promptly depress "start" on the monitor.
8. Record the rates for the two (control and experimental) cuvettes.

† Volume of cell extract may be adjusted so that change in absorbance is between about 0.05 and 0.7 AU in one minute. Any change in cell extract should be accompanied by a change in the DI water in the cocktails. (For example, if 50 µL of cell extract were used, then 260 µL of DI water would be used in the experimental.)

Calculation of Activity

One unit (U) of tyrosine phenol lyase activity is defined as the amount of enzyme required to produce 1.0 µmole of pyruvate in one minute.

1. $dA/dt\ (min^{-1}) = [Rate]_{experimental} - [Rate]_{control} = dA/dt$

2. $$\text{Activity} = \frac{1000 \times TV \times dA/dt}{\varepsilon \times V \times CF}$$

Activity: Volumetric Activity (U/L)
TV: Total volume in cuvette (1000 µL)
V: Volume of cell extract used (100 µL)

ε: Molar extinction coefficient for NADH (6.22 L/mmol for a path length of 1.0 cm)
CF: Concentration factor of cell extract (For example, if a 100 mL sample is concentrated to a 5 mL volume for the French Press, then CF=20)

3. Specific Activity = $\frac{Activity}{Protein\ Concentration}$

Activity: Volumetric Activity, as calculated in #2 above (U/L)
Protein Concentration: Protein concentration, as calculated in protocol **Total Protein Concentration** (mg/L)
Specific Activity: (U/mg protein)

Reference

Notes

The product phenol is a competitive inhibitor of the reaction. Therefore, care must be taken to use only initial rate data.