Alanine Dehydrogenase
April 5, 2005

Summary

Alanine dehydrogenase catalyzes the oxidative deaminiation of L-alanine to pyruvate using NAD as a co-substrate. This protocol describes a direct enzyme assay for determining alanine dehydrogenase activity.

Solutions Required

1. 500 mM glycine/KCl buffer:
   prepared by dissolving 3.75 g glycine and 3.73 g KCl in water to 100 mL.
   adjust to a pH of 10.5 with 20% KOH.

2. 10 mM potassium phosphate buffer pH = 7.0
   prepared by mixing 8 mL of 10 mM KH_2PO_4 and 20 mL of 10 mM K_2HPO_4.
   or prepared by dissolving 0.0194 g KH_2PO_4 and 0.0815 g $K_2HPO_4 \cdot 3H_2O$ in 50 mL water.

3. 1.0 M L-alanine
   can be prepared in stock solution and stored in refrigerator.

4. 4.0 mM NAD^+
   must be prepared fresh

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	500	400
glycine/KCl	300	300
NAD	100	100
alanine	0	100

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. Mix the solutions in this way ten times. (Count!)
7. Promptly depress "start" on the monitor.
8. Record the rates for the two (control and experimental) cuvettes.

† Dilution of the cell extract may be adjusted so that change in absorbance is between about 0.05 and 0.7 AU in one minute. This dilution should be accomplished externally in a microcentrifuge tube (for example, by adding 50 µL of cell extract to 950 µL DI water to achieve a dilution of 20). The volume of 100 µL should always be used in the enzyme assay mixture.

Calculation of Activity

One unit (U) of alanine dehydrogenase 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. $$\text{Activity} = \frac{1000 \times TV \times D \times dA/dt}{\varepsilon \times V \times CF}$$

Activity: Volumetric Activity (U/L)
TV: Total volume in cuvette (1000 µL)
D: Dilution of the cell extract. (For example, if 50 µL of cell extract were add to 950 µL DI water prior to using a volume of cell extract in the assay, then D=20)
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 2 mL volume for the French Press, then CF=50)

3. $$\text{Specific Activity} = \frac{Activity}{Protein\ Concentration} 1$$

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

T. Ohashima, K. Soda (1979) Purification and properties of alanine dehydrogenase from *Bacillus spharicus*, Eur. J. Biochem. 100, 29-39.