Xylose Reductase
June 11, 2001

Summary

Xylitol reductase catalyzes the reduction D-xylose to xylitol using NADPH as a co-substrate. This protocol describes a direct enzyme assay for determining xylitol reductase activity.

Solutions Required

1. 250 mM potassium phosphate buffer pH = 7.0.
   prepared by mixing 36 mL of 250 mM KH_2PO_4 and 45 mL of 250 mM K_2HPO_4.

2. 100 mM 2-mercaptoethanol
   can be prepared in stock solution and stored in refrigerator.

3. 3.4 mM NADPH
   must be prepared fresh

4. 0.5 M D-xylose
   can be prepared in stock solution and stored in refrigerator.

Preparation of Cell Extract

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

1. Centrifuge sufficient cells so that the volume diluted down to 5 mL would give an optical density of 20-30. For example, for a broth of OD=1, use 100 mL. For a broth of OD=10, use 10 mL.
2. After first pelletization of cells, resuspend in 5-15 mL of 4°C potassium phosphate buffer.
3. After second pelletization of cells, resuspend in 5 mL of 4°C potassium phosphate buffer, and break with French Press.

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	200	100
potassium phosphate	600	600
2-mercaptoethanol	100	100
NADPH	50	50
xylose	0	100

2. Directly from the ice when ready to commence the assay, place the two cuvettes (each containing 950 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 50 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 20 µL of cell extract were used, then 130 µL of DI water would be used in the experimental.)

Calculation of Activity

One unit (U) of xylose reductase activity is defined as the amount of enzyme required to produce 1.0 µmole of xylitol 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 (50 µL)
ε: Molar extinction coefficient for NADPH (6.22 L/mmol for a path length of 1.0 cm)

CF: Dilution 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

S.-I. Yokoyama, T. Suzuki, K. Kawai, H. Horitsu, K. Takamizawa (1995) Purification, Characterization and Structure Analysis of NADPH-Dependent D-Xylose Reductase from *Candida tropicalis*. J. Ferm. Bioeng. 79 (3) 217-223.