Urease (URE)

from jack bean

Urea amidohydrolase, EC 3.5.1.5

Urea + H₂O → CO₂ + 2 NH₃

SPECIFICATION

- Appearance: white lyophilizate
- Activity: ≥100 U/mg lyophilizate
- Storage: at −20°C

PROPERTIES

- Molecular weight: ca. 480 kDa (gel filtration)
- Structure: 8 subunits (containing nickel)
- Isoelectric point: 5.0–5.1
- Michaelis constant: 1.05×10⁻² M (urea)
- pH Optimum: ca. 8.0 (Fig. 1)
- pH Stability: 5.0–10.0 (Fig. 2)
- Optimum temperature: 60°C (Fig. 3)
- Thermal stability: below 50°C (Fig. 4)
- Inhibitors: heavy metal ions, Na⁺, K⁺, NH₄⁺, suramin, thiourea
- Specificity: specific for urea
ASSAY PROCEDURE

Principle

\[
\text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{urease}} \text{CO}_2 + 2 \text{NH}_3
\]

\[
2 \text{NH}_3 + 2 \alpha\text{-Ketoglutarate} + 2 \text{NADH} + 2 \text{H}^+ \xrightarrow{\text{glutamate dehydrogenase}} 2 \text{L-Glutamate} + 2 \text{NAD}^+
\]

The disappearance of NADH is measured spectrophotometrically at 340 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 2 μmol of ammonia per min at 25°C and pH 8.0 under the conditions described below.

One unit under these conditions is equivalent to 2.4 Nessler units.

Reagents

A. Potassium phosphate buffer, 50 mM; pH 7.4: mix 50 mM KH₂PO₄ solution and 50 mM K₂HPO₄ solution to make a pH 7.4 solution.
B. Buffered water (potassium phosphate buffer, 5.0 mM; pH 7.4): dilute potassium phosphate buffer (Reagent A) to 10-fold volume with distilled water.
C. NADH solution, 8.5 mM: 30 mg of NADH/5 ml of buffered water (Reagent B).
D. \(\alpha\)-Ketoglutarate solution, 25 mM: dissolve 91.3 mg of \(\alpha\)-ketoglutarate in 10 ml of distilled water, adjust to pH 5.0 with 2 M NaOH solution and dilute with distilled water to 25 ml.
E. Urea solution, 0.3 M: 90 mg of urea/5 ml of distilled water. (Prepare freshly)
F. Glutamate dehydrogenase (GLDH) solution: 250 U/ml of potassium phosphate buffer (Reagent A).
G. Enzyme dilution buffer: dissolve 6.06 g of Tris(hydroxymethyl)aminomethane and 0.37 g of EDTA·Na₂·2H₂O in 800 ml of distilled water, adjust to pH 8.0 with 6.0 M HCl and dilute with distilled water to 1000 ml.

Sample: dissolve the lyophilized enzyme to a volume activity of 0.10–0.25 U/ml with ice-cold enzyme dilution buffer (Reagent G) immediately before measurement.

Procedure

1. Pipette the following reagents into a cuvette (light path: 1 cm).
   - 0.1 ml NADH solution (Reagent C)
   - 0.1 ml \(\alpha\)-Ketoglutarate solution (Reagent D)
   - 0.1 ml GLDH solution (Reagent F)
   - 2.5 ml Enzyme dilution buffer (Reagent G)
2. Equilibrate at 25°C for about 5 min.
3. Add 0.1 ml of sample and mix.
4. Add 0.1 ml of urea solution (Reagent E) and mix.
5. Record the decrease of absorbance at 340 nm in a spectrophotometer thermostated at 25°C, and calculate the \(\Delta A\) per min using the linear portion of the curve (\(\Delta A_o\)).

The blank solution is prepared by adding enzyme dilution buffer (Reagent G) instead of sample (\(\Delta A_b\)).
**Calculation**

Activity can be calculated by using the following formula:

\[
\text{Volume activity (U/ml) = } \frac{(\Delta A_6 - \Delta A_3) \times 3.0 \text{ (ml)} \times df}{6.2 \times 0.1 \text{ (ml)} \times 2} = \Delta A \times 2.42 \times df
\]

\[
\text{Weight activity (U/mg) = } (\text{U/ml}) \times \frac{1}{C}
\]

6.2 : Millimolar extinction coefficient of NADH at 340 nm (cm\(^2\)/\(\mu\)mol)
2 : Factor to allow for 2 mol of ammonia produced from 1 \(\mu\)mol of urea
df : Dilution factor
C : Content of urease preparation in sample (mg/ml)

**APPLICATIONS**

The enzyme is useful for the determination of urea in clinical analysis.

\[
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\]

\[
2 \text{NH}_3 + 2 \alpha\text{-Ketoglutarate} + 2 \text{NADH} + 2 \text{H}^+ \xrightarrow{\text{glutamate dehydrogenase}} 2 \text{L-Glutamate} + 2 \text{NAD}^+
\]

**REFERENCES**

**EXPERIMENTAL DATA**

**Fig. 1  pH Optimum**

- Relative activity (%)
- **pH**
- $\triangle$: 50 mM acetate buffer
- $\bigcirc$: 50 mM phosphate buffer
- $\vartriangle$: 50 mM TAPS-NaOH buffer
- $\blacksquare$: 50 mM CHES-NaOH buffer

**Fig. 2  pH Stability**

- Residual activity (%)
- **pH**
- Treatment: 5°C, 2 h
- $\triangle$: 50 mM acetate buffer
- $\bigcirc$: 50 mM phosphate buffer
- $\vartriangle$: 50 mM TAPS-NaOH buffer
- $\blacksquare$: 50 mM CHES-NaOH buffer

**Fig. 3  Optimum temperature**

- Relative activity (%)
- **°C**
- Buffer: 50 mM Tris-HCl buffer, pH 8.0, containing 1 mM EDTA

**Fig. 4  Thermal stability**

- Residual activity (%)
- **°C**
- Treatment: 20 mM phosphate buffer, pH 7.0, containing 1 mM EDTA, 60 min