

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

# **Product Information**

Urease, Type C-3 from Canavalia ensiformis (Jack Bean)

Catalog Number **U0251** Storage Temperature –20 °C

E.C. 3.5.1.5 CAS RN 9002-13-5

Synonym: Jack Bean Urease

# **Product Description**

Urease is involved in purine metabolism and the urea cycle. It catalyzes the hydrolysis of urea to produce ammonia and carbon dioxide:

Urease

Urea + H₂O

 $\rightarrow$ 

CO<sub>2</sub> + 2 NH<sub>3</sub>

Hydroxyurea is also a substrate of the enzyme.<sup>1</sup>

Jack bean urease was the first enzyme to be crystallized and the first enzyme found to contain nickel. It is a multi-subunit enzyme, consisting of 91 kDa subunits in three protein forms. The major protein form has a molecular mass range of 440–480 kDa and two lesser forms have molecular mass ranges of 230–260 kDa and 660–740 kDa.<sup>2,3</sup>

Isoelectric point:4 5.0-5.2

Optimal pH:2 7.4

Optimal temperature: 60 °C Urease begins to denature at temperatures above 45 °C for 60 minutes.

K<sub>M</sub>:<sup>2</sup> 1.3 mM (in Tris HCl)

Inhibitors:
2-mercaptoethanol<sup>5</sup>
acetohydroxamate<sup>6</sup>
EDTA<sup>7</sup>
phosphoramidate<sup>5</sup>
fluoride ion<sup>5</sup>
1,4-benzoquinone
2,5-dimethyl-1,4-benzoquinone<sup>8</sup>

This product is purified by crystallization and is supplied as a powder.

Specific activity: ≥600,000 units/g solid

Unit definition: one unit will liberate 1.0  $\mu$ mole of NH<sub>3</sub> from urea per minute at pH 7.0 at 25 °C. One unit is equivalent to 1.0 I.U. or 0.054 Sumner unit (1.0 mg ammonia nitrogen released in 5 minutes at pH 7.0 at 20 °C)

Sigma's titrimetric assay uses a 1.10 ml reaction mix. The final concentrations are 684 mM sodium phosphate, 455 mM urea, 0.05% (w/v) bovine serum albumin and 25–50 units of urease.

A FTIR method used to monitor either the disappearance of substrate or the appearance of product has been published.<sup>9</sup>

#### **Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### **Preparation Instructions**

The enzyme is soluble in 0.2 M sodium phosphate buffer, pH 7.0, (1 mg/ml) yielding a colorless to light yellow solution with a haze. The following buffers have been shown not to inhibit urease activity: MES, HEPES, and CHES.<sup>2</sup>

# Storage/Stability

The recommended storage temperature is -20 °C.

#### References

- Fishbein, W., and Carbone, P., J. Bio. Chem., 265, 9464 (1990).
- Cesareo, S.D., and Langton, S.R., Kinetic properties of *Helicobacter pylori* urease compared with jack bean urease. FEMS Micobiol. Lett., 78, 15-21 (1992).
- 3. Krajewska, B., and Ciurli, S., Jack Bean (*Canavalia ensiformis*) urease. Probing acid-base groups of the active site by pH variation. Plant Physiol. Biochem., **43**, 651-58 (2005).
- 4. Sumner, J.B., and Hand, D.B., The isoelectric point of crystalline urease. J. Am. Chem. Soc., **51**, 1255-60 (1929).
- Dixon, N.E. et al., Jack Bean urease (EC 3.5.1.5).
   III. The involvement of active-site nickel ion in inhibition by beta-mercaptoethanol, phosphoramidate, and fluoride. Can. J. Biochem., 58, 481-488 (1980).

- Dixon, N.E. et al., Metal ions in enzymes using ammonia or amides. Science, 191, 1144-1150 (1976).
- 7. Dixon, N.E. *et al.*, Jack been urease (EC 3.5.1.5). II. The relationship between nickel, enzymatic activity, and the "abnormal" ultraviolet spectrum. The nickel content of jack beans. Can. J. Biochem., **58**, 474-480 (1980).
- Zaborska, W. et al., Inhibition of jack bean urease by 1,4-benzoquinone and 2,5-dimethyl-1,4-benzoquinone. Evaluation of the inhibition mechanism. J. Enzyme Inhib. Med. Chem., 17, 247-53 (2002).
- 9. Karmali, K. et al., The use of Fourier transform infrared spectroscopy to assay for urease from *Pseudomonas aeruginosa* and *Canavalia ensiformis*. Anal. Biochem., **331**, 115-21 (2004).

GY,KAD,RBG,JWM,MAM 03/14-1