上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

L-Arginase

(L-Arginine amidinohydrolase; EC 3.5.3.1)

L-Arginase causes the following reaction:

$$\text{L-arginine} + H_2O \xrightarrow{\text{L-Arginase}} \text{L-ornithine} + \text{urea}$$

The quarternary structure of native rat liver arginase has been described by Hirsch-Kolb, H & Greenberg, D.M.," Molecular characterisitics of rat liver arginase", J. Biol Chem., 243, 6123-6129 and Baranczyk-Kuzma A., Porembska Z. & Mochnacka, I., Acta Biochim Polon, 23, 151-163. EDTA treatment dissociated the enzyme into inactive subunits of 30,000 daltons each. Addition of Mn2+ ions restored the activity and caused reassociation of subunits to the natve form of 120,000 daltons.

ASSAY

One unit of enzyme activity is defined as that amount of enzyme that causes the hydrolysis of one micromole of L-arginine per minute at 37°C and pH 9.5.

REAGENTS

1. 0.2 M L-arginine solution: 1.05 g L-arginine monochloride/25 ml, adjusted to pH 9.5 with 1 N NaOH.
2. 12.5 mM Urea standard sol'n. (75 mg urea/100 ml)
3. 0.084 N Sulfuric acid (2.32 ml conc. H_2SO_4/1000 ml)
4. 0.3 M Sodium tungstate, pH 7.0: 10 g $Na_2WO_4 \bullet 2H_2O$/100 ml, pH adjusted to 7.0 with 1 N H_2SO_4
5. 0.03 M Tungstic acid solution: Mix 9 parts H_2SO_4 (3) with 1 part sodium tungstate solution (4). Prepare fresh prior to assay.
6. 60% (v/v) Phosphoric acid (60 ml conc. H_3PO_4, approx. 85-87%/100 ml).
7. 60 mM Diacetylmonoxime/3.3 mM thiosemicarbazide reagent: Mix 600 mg diacetylmonoxime + thiosemicarbazide/100ml. Mix 10 parts H_3PO_4 (6) with 2 parts solution immediately prior to assay.
8. 10 mM Manganese-maleate buffer: 10 mM Mn^{2+}, 10 mM maleate (116 mg maleic acid anhydride/100 ml,, adjusted to pH 9.7 with 0.1 N NaOH; add 0.5 ml 2 M $MnSO_4$ solution and adjust to pH 7.5 with 0.1 M H_2SO_4.
9. L-Arginase: 1 mg /ml solution in 10 mM Manganese-maleate buffer (8) diluted to 1:500 dilution.

PROCEDURE

1. Set up water bath at 37°C.
2. Into two test tubes, pipette the following reagents:

	STANDARD	SAMPLE
Arginine sol'n (1)	0.20 ml	0.20 ml
Distilled water		0.20 ml
Urea standard sol'n. (2)	0.20 ml	
Diluted enzyme sol'n (9)		0.10 ml

3. Incubate at 37°C for 30 minutes.
4. Then add the following:

Tungstic acid solution (5)	4.50 ml	4.50 ml
Diluted enzyme sol'n (9)	0.10 ml	

5. Allow to stand for 5 min. at room temperature and centrifuge the precipitate and add 0.2 ml of supernatant and 5 ml of Reagent 7 to develop the color to standard and blank. Heat in boiling water for 30 min. and measure the ΔE_{546nm} of sample and ΔE_{546nm} of standard.

CALCULATION

$$\text{Activity (U/mg)} = \frac{(\Delta E_{546nm/min})(2.5)}{(\Delta E_{546nm/min})(30)(0.1)(\text{mg Enz./ml})}$$