ENZYMES

Uricase

ORIGIN *Bacillus fastidiosus*

CAT# URIC-70-1701
EC# 1.7.3.3

SPECIFICATIONS

Appearance	White/off white free flowing powder	
Powder Activity	>10 U/mg powder at 37°C	
Specific Activity	>10 U/mg protein at 37°C	
Contaminants	Catalase	<1%
	Cholesterol Oxidase	<0.005%
	Glucose Oxidase	<0.005%

APPLICATION

Uricase can be used (in test strips or other clinical chemistry formats) to determine the level of uric acid in blood for clinical diagnosis.

UNIT DEFINITION

One unit of activity is defined as the amount of enzyme that will catalyse the oxidation of 1.0 micromole of uric acid per minute at 37°C under the standard assay method conditions. Refer to Table 1 for guidance on factors to adjust according to temperature of assay.

TABLE 1: TEMPERATURE FACTORS FOR UNIT CONVERSION

Note: Temperature can influence the level of available oxygen in the reaction mixture.

ASSAY TEMPERATURE	FACTOR RELATIVE TO 37°C RESULT
25°C	0.23
30°C	0.48
37°C	1.00
45°C	1.16

ASSAY PRINCIPLE

Uricase catalyses the following reaction:

$$\text{Uric acid} + 2H_2O_2 + O_2 \xrightarrow{\text{Uricase}} \text{Allantoin} + H_2O_2 + CO_2$$

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CHARACTERISTICS

Uricase is a highly purified product and is formulated with BSA as stabiliser. Its main characteristics are as follows:

Molecular Weight[1]:	38kD
Km (Eadie-Hofstee):	2 x 10^{-4} M (Uric acid)
Optimum pH (Fig. 1):	pH 7.0 (phosphate buffer)
Optimum Temperature (Fig. 2):	45°C
pH Stability (Fig. 3):	pH 5.5 to -10.0 (25°C for 20 hours)
Thermal Stability (Fig. 4):	Stable at 50°C and below

TABLE 2: SUBSTRATE SPECIFICITY

Substrate specificity was tested in-house by replacing uric acid with alternative substrates in the assay i.e. at 2.3 mM concentration.

ASSAY TEMPERATURE	% OF URIC ACID ACTIVITY
Uric acid	100
8-Azaxanthine	0
Oxonic acid	0

FIGURE 1: OPTIMUM pH

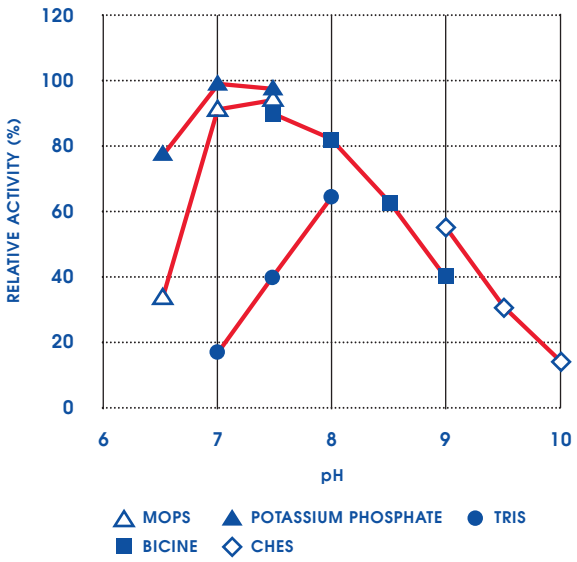
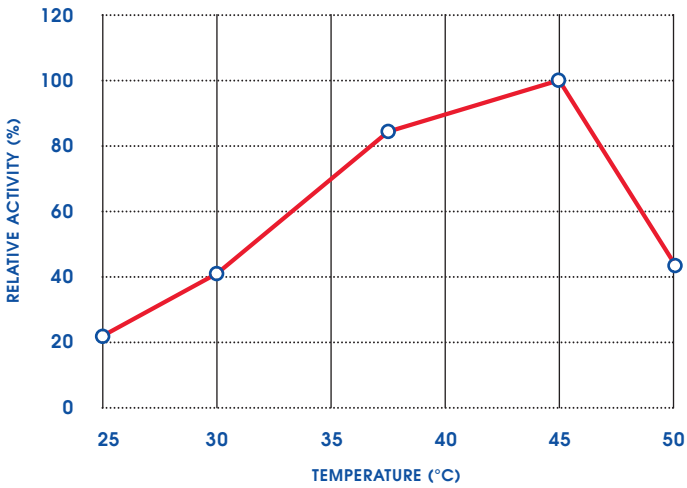


FIGURE 2: OPTIMUM TEMPERATURE



Note: Uric acid is particularly insoluble under acidic conditions and so determination of uricase activity below a pH of 6.5 was not deemed practicable.

FIGURE 3: pH STABILITY (25°C FOR 20 HOURS)

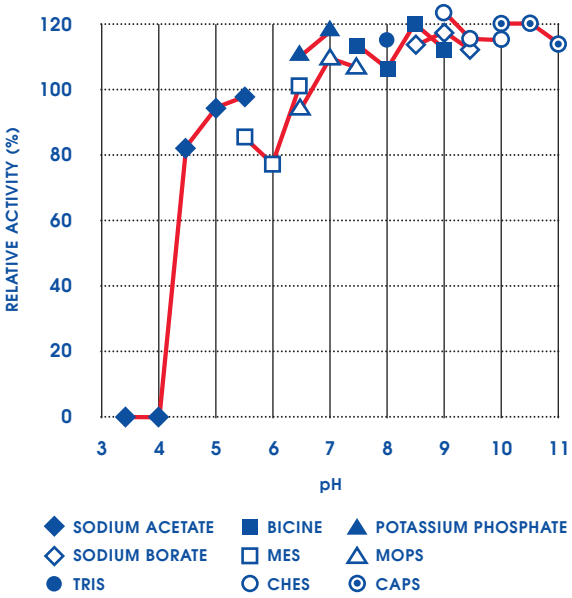
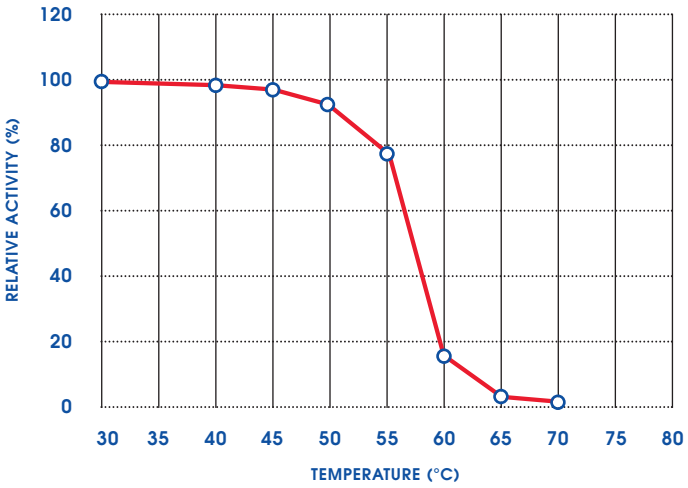


FIGURE 4: THERMAL STABILITY (pH 7.0 FOR 15 MINUTES)



(1) Bongaerts,G.P.A. et al. (1978) Uricase of Bacillus Fastidiosus: Properties and regulation of synthesis. Biochimica et Biophysica Acta – Enzymology, 527 (2) pp.348-358.

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