SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE (EC 3.3.1.1)

PRINCIPLE:

$$\text{S-Adenosyl-L-Homocysteine} + H_2O \xrightarrow{\text{SAHase}} \text{Adenosine} + \text{L-Homocysteine}$$

$$\text{Adenosine} + H_2O \xrightarrow{\text{Adenosine Deaminase}} \text{Inosine} + NH_3$$

$$\text{Inosine} + \text{Orthophosphate} \xrightarrow{\text{Nucleoside Phosphorylase}} \text{Hypoxanthine} + \text{Ribose-1-Phosphate}$$

$$\text{Hypoxanthine} \xrightarrow{\text{Xanthine Oxidase}} \text{Xanthine}$$

$$\text{Xanthine} + H_2O + O_2 \xrightarrow{\text{Xanthine Oxidase}} \text{Uric Acid} + H_2O_2$$

Abbreviations used:
SAHase = S-Adenosyl-L-Homocysteine Hydrolase

CONDITIONS: T = 37°C, pH = 7.2, A_{292nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 7.2 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.2 at 37°C with 1 M KOH.)

B. 5.2 mM S-Adenosyl-L-Homocysteine Solution (SAH)
(Prepare 10 ml in deionized water using S-Adenosyl-L-Homocysteine, Sigma Prod. No. A-9384.)

C. Adenosine Deaminase Enzyme Solution (ADA)
(Use Adenosine Deaminase, Type VI from Calf Intestinal Mucosa, Sigma Prod. No. A-1155, undiluted, approximately 1500 u/ml.)

D. Xanthine Oxidase Enzyme Solution (XO)
(Use Xanthine Oxidase, Grade I from Buttermilk, Sigma Prod. No. X-1875, undiluted, approximately 20 u/ml.)

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REAGENTS: (continued)

E. Nucleoside Phosphorylase Enzyme Solution (NP)
(Use Nucleoside Phosphorylase, from Calf Spleen, Sigma Prod. No. N-3003, undiluted, approximately 120 u/ml.)

F. S-Adenosyl-L-Homocysteine Hydrolase Enzyme Solution (SAHase)
(Use undiluted, containing 7.0 - 20 units/ml)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reagent A (Buffer)	3.00	3.00
Reagent B (SAH)	0.03	0.03
Reagent C (ADA)	0.03	0.03
Reagent D (XO)	0.03	0.03
Reagent E (NP)	0.03	0.03

Mix by inversion and equilibrate to 37°C. Monitor the A_{292nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (SAHase)	0.30	------
Deionized Water	------	0.30

Immediately mix by inversion and record the increase in A_{292nm} for approximately 10 minutes. Obtain the ΔA_{292nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{292nm}/\text{min Test} - \Delta A_{292nm}/\text{min Blank})(3.42)(df)}{(0.0121)(0.1)}$$

3.42 = Volume (in milliliter) of assay
0.0121 = Micromolar extinction coefficient of Uric Acid
0.1 = Volume (in milliliter) of enzyme used

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

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UNIT DEFINITION:

One unit will hydrolyze 1.0 nanomole of S-adenosyl-L-homocysteine to adenosine and L-homocysteine per minute at pH 7.2 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.42 ml reaction mix, the final concentrations are 93 mM potassium phosphate, 48.4 μM S-adenosyl-L-homocysteine, 45 units adenosine deaminase, 0.6 unit xanthine oxidase, 3.6 units nucleoside phosphorylase, and 0.7 - 2.0 units S-adenosyl-L-homocysteine lyase.

NOTES:

1. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.