SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of ADENOSINE DEAMINASE (EC 3.5.4.4)

PRINCIPLE:

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

CONDITIONS: T = 25°C, pH = 7.5, A_{265nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 7.5 at 25°C
(Prepare 150 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.5 at 25°C with 1 M KOH.)

B. 1.35 mM Adenosine Solution, pH 7.0 at 25°C[1]
(Prepare 10 ml in deionized water using Adenosine, Sigma Prod. No. A-9251. Adjust to pH 7.0 at 25°C with 10 mM NaOH.)

C. 0.1% (w/v) Bovine Serum Albumin Solution (BSA)
(Prepare 50 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503.)

D. Adenosine Deaminase Enzyme Solution
(Immediately before use, prepare a solution containing 0.20 - 0.40 unit/ml of Adenosine Deaminase in cold Reagent C.)

PROCEDURE:

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

	Test	Blank
Deionized Water	1.30	1.30
Reagent A (Buffer)	1.50	1.50
Reagent B (Adenosine)	0.10	0.10

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 25°C. Monitor the A_{265nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

	Test	Blank
Reagent C (BSA)	------	0.10
Reagent D (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the decrease in the A_{265nm} for approximately 5 minutes. Obtain the ΔA_{265nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{265nm}/\text{min Test} - \Delta A_{265nm}/\text{min Blank})(3)(df)}{(8.1)\,(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution Factor
8.1 = Millimolar extinction coefficient of adenosine at 265 nm
0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will deaminate 1.0 μmole of adenosine to inosine per minute at pH 7.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 53.3 mM potassium phosphate, 0.045 mM adenosine, 0.003% (w/v) bovine serum albumin and 0.02 - 0.04 unit of adenosine deaminase.

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REFERENCE:

Murphy, J., Baker, D.C., Behling, C. and Turner, R.A. (1982) *Anal. Biochemistry* **122**, 328-337

Bergmeyer, H.U. (1983) in *Methods of Enzymatic Analysis*,
3rd ed., Vol. 2, pp 135-136, Verlag Chemie, Deerfield Beach, FL

NOTES:

1. Substrate concentration should not be significantly increased above that listed in the procedure. Higher concentrations result in a deviation from Beers Law leading to errors in the rate determinations according to Murphy et al. (1982).

2. This assay is based on the cited references.

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