Enzymatic Assay of KANAMYCIN 6'-ACETYLTRANSFERASE (EC 2.3.1.55)

PRINCIPLE:

Acetyl-CoA + Kanamycin $\xrightarrow{KAT}$ CoA + N^6'-Acetylkanamycin

CoA + DTNB ———> TNB + CoA Derivative

Abbreviations used:
Acetyl-CoA = Acetyl Coenzyme A
KAT = Kanamycin 6'-Acetyltransferase
CoA = Coenzyme A
DTNB = 5,5'-Dithio-bis-(2-Nitrobenzoic Acid)
TNB = 5-Thio-2-Nitrobenzoic Acid

CONDITIONS: T = 37°C, pH = 5.7, A_{412nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM MES Buffer, pH 5.7 at 37°C
(Prepare 100 ml in deionized water using MES, Free Acid, Sigma Prod. No. M-8250. Adjust to pH 5.7 at 37°C with
1 M NaOH.)

B. 17 mM 5,5'-Dithio-bis(2-Nitrobenzoic Acid) (DTNB)
(Immediately before use, prepare 3 ml in deionized water using 5,5'-Dithio-bis(2-Nitrobenzoic Acid), Sigma Prod. No. D-8130. Solubilize by the addition of a minimal volume of 0.1 M NaOH. The color of this solution must be a very pale yellow.)

C. 5 mM Acetyl Coenzyme A Solution (Acet CoA)
(Prepare 10 ml in deionized water using Acetyl Coenzyme A, Sodium Salt, Sigma Prod. No. A-2056.)

D. 0.3% (w/v) Kanamycin Sulfate Solution (KS)
(Prepare 1 ml in deionized water using Kanamycin, Monosulfate, Sigma Prod. No. K-4000.)

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REAGENTS:

E. 10 mM Tris HCl, pH 7.8 at 37°C (Enz Dil)
(Prepare 50 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.8 at 37°C with 1 M HCl.)

F. Kanamycin 6'-Acetyltransferase Enzyme Solution
(Immediately before use, prepare a solution containing 50 units/ml of Kanamycin 6'-Acetyltransferase in cold Reagent E.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent B (DTNB)	3.00
Reagent C (Acet CoA)	1.00
Reagent A (Buffer)	46.00

Mix by swirling.

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

	Test	Blank
Reaction Cocktail	2.90	2.90
Reagent F (Enzyme Solution)	0.05	------
Reagent E (Enz Dil)	------	0.05

Mix by inversion and equilibrate to 37°C. Monitor the A_{412nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (KS)	0.05	0.05

Immediately mix by inversion and record the increase in A_{412nm} for approximately 5 minutes. Obtain the ΔA_{412nm}/minute using the maximum linear rate for both the Test and Blank.

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CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{412nm}/\text{min Test} - \text{r } A_{412nm}/\text{min Blank})(3)(df)}{(0.0136)(0.05)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
0.0136 = µmolar extinction coefficient for 5-Thio-2-Nitrobenzoic Acid at 412nm
0.05 = Volume (in milliliter) 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 convert 1.0 nanomole of kanamycin and acetyl-CoA to kanamycin 6-acetate and CoA per minute at pH 5.7 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final concentrations are 89 mM MES, 1 mM 5,5'-dithio-bis-(2-nitrobenzoic acid), 0.1 mM acetyl coenzyme A, 0.005% (w/v) kanamycin sulfate, 0.2 mM Tris, and 2.5 units kanamycin 6'-acetyltransferase.

REFERENCES:

Benveniste, R. and Davies, J. (1971) *Biochemistry* **10**, 1787-1796

Silverstein, R.M. (1975) *Analytical Biochemistry* **63**, 281-282

NOTES:

1. The extinction coefficient of TNB is described in Silverstein, R.M. (1975.).

2. This assay is based on the cited references.

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