SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of δ-AMINOLEVULINATE DEHYDRATASE (EC 4.2.1.24)

PRINCIPLE:

2 δ-Aminolevulinic Acid $\xrightarrow{AVD}$ > Porphobilinogen + $2H_2O$

Abbreviation:
AVD = δ-Aminolevulinate Dehydratase

CONDITIONS: T = 37°C, pH = 6.5, A_{555nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 100 mM Potassium Phosphate Buffer with 20 mM Dithiothreitol, pH 6.5 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, and DL-Dithiothreitol, Sigma Prod. No. D-0632. Adjust to pH 6.5 at 37°C with 1 M KOH.)

B. 50 mM δ-Aminolevulinic Acid Solution (AV Acid)
(Prepare 5 ml in Reagent A using δ-Aminolevulinic Acid, Hydrochloride, Sigma Prod. No. A-3785.)

C. 0.16 mM Porphobilinogen Solution (Porph)
(Prepare 10 ml in Reagent A using Porphobilinogen, Sigma Prod. No. P-1134.)

D. 10% (v/v) Trichloroacetic Acid with 100 mM Mercuric Chloride Solution (TCA)
(Prepare 15 ml in deionized water using Trichloroacetic Acid Solution, 6.1 N, approximately 100% (w/v), Sigma Stock. No. 490-10, and Mercuric Chloride, Sigma Prod. No. M-6529.)

E. Ehrlich's Color Reagent (ECR)
(Prepare by adding 1 g of p-Dimethylaminobenzaldehyde, Sigma Prod. No. D-2004 to 42 ml of Acetic Acid, Glacial, Sigma Prod. No. A-6283. Then add 8 ml of Perchloric Acid, Sigma Stock No. 24425-2.)

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REAGENTS: (continued)

F. δ-Aminolevulinate Dehydratase (Enzyme Soln)
(Immediately before use, prepare a solution containing 0.15 unit/ml in cold Reagent A.)

PROCEDURE:

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

	Test1	Test2	Test3	Blank	Std1	Std2	Std3	Std4	Std5	Std Blank
Reagent A (Buffer)	0.70	0.65	0.60	0.90	0.80	0.75	0.70	0.65	0.60	1.00
Reagent F (Enzyme Soln)	0.20	0.25	0.30	0.10	------	------	------	------	------	------

Mix by vortexing and equilibrate at 37°C for 10 minutes (time required for enzyme activation). Then add:

Reagent C (Porph)	------	------	------	------	0.20	0.25	0.30	0.35	0.40	------
Reagent B (AV Acid)	0.10	0.10	0.10	------	------	------	------	------	------	------

Mix by vortexing and incubate at 37°C for 60 minutes. Then add:

Reagent D (TCA)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Mix by vortexing and centrifuge to clarify. Pipette (in milliliters) the following into suitable cuvettes:

Test Supernatant	0.50	0.50	0.50	------	------	------	------	------	------	------
Blank Supernatant	------	------	0.50	------	------	------	------	------	------	------

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PROCEDURE: (continued)

	Test1	**Test2**	**Test3**	**Blank**	Std1	Std2	Std3	Std4	Std5	Std Blank
Std Supernatant	------	------	------	------	0.50	0.50	0.50	0.50	0.50	------
Std Blk Supernatant	------	------	------	------	------	------	------	------	------	0.50
Reagent E (ECR)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Mix by inversion and let the precipitate settle.[1] Read the A_{555nm} for the Test, Blank, Standards, and Standard Blank after 10 minutes.

CALCULATIONS:

Standard Curve:

ΔA_{555nm} Standard = A_{555nm} Standard - A_{555nm} Standard Blank

Prepare a Standard Curve by plotting the ΔA_{555nm} Standard vs the µmoles of porphobilinogen.

Sample Determination:

ΔA_{555nm} Test = A_{555nm} Test - A_{555nm} Blank

Determine the µmoles of porphobilinogen produced using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{mole of porphobilinogen produced})}{(0.25)}$$

0.25 = Volume (in milliliter) of enzyme used (may also be 0.20 or 0.30 depending upon the enzyme volume)

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

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

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UNIT DEFINITION:

One unit will produce 1.0 µmole of porphobilinogen from δ-aminolevulinic acid in 60 minutes at pH 6.5 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 100 mM potassium phosphate, 20 mM dithiothreitol, 5 mM δ-aminolevulinic acid, and 0.03 - 0.045 unit δ-aminolevulinate dehydratase.

REFERENCES:

Jordan, P.M. and Seehra, J.S. *Methods in Enzymology*, Volume 123, 427-434

NOTES:

1. This precipitate does not interfere with the assay and the solution is not centrifuged after formation of the precipitate. This eliminates contaminating additional laboratory glassware with mercuric chloride.

2. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.