

Citrate Synthase

Reference-

1) Bergmeyer- Methods of Enzymatic Analysis- Vol. 1, p443

Principle-



Acetyl CoA has an absorption band at 232nm due to the thioester band. As the assay proceeds- and the Acetyl CoA is used there is a decrease in absorption.

Acetyl CoA $E = 5.4 \text{ cm}^2/\mu\text{mole}$

Solutions- make the following stock solutions-

1. 1M Tris HCl pH 8.1 (50 Base/50 Acid)-

Trizma Base- (Sigma T-1503) MW 121.1 Weigh 60.55 gms and place in a 1 liter beaker. Weigh 78.8 gms of Trizma HCl (Sigma T-7149) MW 157.6 and add to the same beaker. Bring volume to app. 800 mls and check the ph. If minor adjustment is necessary, use either 1N HCl, or NaOH to bring the ph to 8.1. Adjust the final volume to 1Liter. Store as 250 ml aliquots at -20°C. Thaw before use and keep on ice.

2. 0.5 M OxalaloAcetic Acid- Sigma O-4126 MW 132.1. Weigh 132mgs and dissolve in 2 mls. Store at -80°C .

3. 0.2M Acetyl CoA- Sigma A-2181 FW-809.6. Weigh 323 mgs and dissolve in 2 mls water. Store at -80°C.

Final conc.	Stock conc.	Lot No.	Volume/10 ml
100 mM Tris pH 8.1	1M		1.0ml
0.17mM OAA	0.5M		3.4μL
0.2M AcCoA	0.2M		10 μL

Protocol (USE QUARTZ CUVETTES)

#	AR	Med	1:10 Homog	1'	2'	5'	10'	10-2/8'
1.	Blk	1 ml	5μL					
2.	Blk	1 ml	5μL					
3.		1 ml	5μL					
4.		1 ml	5μL					

$\mu\text{moles}/\text{min}/\text{gm} =$

$$\frac{\text{net o.d.}/\text{min} \times 1000 \times \text{T.V.}(1.005 \text{ ml})}{5440 \times \text{mgs in assay}(0.5\text{mg})}$$

Updated 2.27.09 RC

Approved Dr. Alan Pestronk