

**Enzymatic Assay of MALATE SYNTHASE  
(EC 4.1.3.2)**

**PRINCIPLE:**

Acetyl CoA + H<sub>2</sub>O + Glyoxylate  $\xrightarrow{\text{Malate Synthase}}$  L-Malate + CoA

CoA + DTNB  $\longrightarrow$  TNB + CoA derivative

Abbreviations used:

Acetyl CoA = Acetyl Coenzyme A

CoA = Coenzyme A

DTNB = 5,5'-Dithio-bis(2-Nitrobenzoic Acid)

TNB = 5-Thio-2-Nitrobenzoic Acid

**CONDITIONS:** T = 30°C, pH 8.0, A<sub>412nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 50 mM Imidazole Buffer, pH 8.0 at 30°C  
(Prepare 100 ml in deionized water using Imidazole, Sigma Prod. No. I-0125. Adjust to pH 8.0 at 30°C with 1 M HCl.)
- B. 100 mM Magnesium Chloride Solution (MgCl<sub>2</sub>)  
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- C. 2.5 mM Acetyl CoA Solution (Acetyl CoA)  
(Prepare 1 ml in deionized water using Acetyl Coenzyme A (C2:0), Sodium Salt, Sigma Prod. No. A-2056.)
- D. 10 mM Glyoxylic Acid Solution (Glyoxylic Acid)  
(Prepare 10 ml in deionized water using Glyoxylic Acid, Sodium Salt, Monohydrate, Sigma Prod. No. G-4502.)
- E. 95% (v/v) Ethanol (EtOH)  
(Prepare 10 ml in deionized water using 200 Proof USP Ethyl Alcohol, available from Quantum Chemical Company.)
- F. 2 mM 5,5'-Dithio-bis(2-Nitrobenzoic Acid) Solution (DTNB)

(Prepare 10 ml in Reagent E using 5,5'-Dithio-bis(2-Nitrobenzoic Acid), Sigma Prod. No. D-8130.)

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**REAGENTS:** (continued)

G. Malate Synthase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.07 - 0.09 unit/ml of Malate Synthase in cold Reagent A.)

**PROCEDURE:**

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

	Test	Blank
Reagent A (Buffer)	0.50	0.50
Reagent B (MgCl <sub>2</sub> )	0.10	0.10
Reagent C (Acetyl CoA)	0.10	0.10
Reagent D (Glyoxylic Acid)	0.10	0.10
Reagent F (DTNB)	0.10	0.10

Mix by inversion and equilibrate to 30°C. Monitor the A<sub>412nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent G (Enzyme Solution)	0.10	-----
Reagent A (Buffer)	-----	0.10

Immediately mix by inversion and record the increase in A<sub>412nm</sub> for approximately 5 minutes. Obtain the r A<sub>412nm</sub>/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\text{r } A_{412\text{nm}}/\text{min Test} - \text{r } A_{412\text{nm}}/\text{min Blank})(1)(\text{df})}{(13.6)(0.1)}$$

1 = Volume (in milliliter) of assay  
df = Dilution factor  
13.6 = Millimolar extinction coefficient for TNB  
at 412 nm  
0.1 = Volume (in milliliter) of assay

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**CALCULATIONS:** (continued)

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

**UNIT DEFINITION:**

One unit will catalyze the cleavage of 1.0  $\mu$ mole of acetyl-CoA per minute at pH 8.0 at 30°C, in the presence of glyoxylate.

**FINAL CONCENTRATIONS:**

In a 1.00 ml reaction mix, the final concentrations are 30 mM imidazole, 10 mM magnesium chloride, 0.25 mM acetyl-CoA, 1 mM glyoxylic acid, 0.2 mM 5,5'-dithio-bis(2-nitrobenzoic Acid), and 0.007-0.009 unit malate synthase.

**REFERENCE:**

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

Chell, R.M., Sundaram, T.K., and Wilkinson, A.E. (1978), *Biochemical Journal* **173**, 165-177

**NOTES:**

1. This assay is based on the cited references.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**