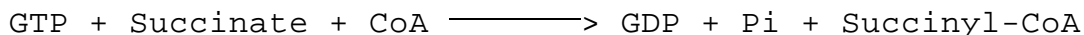


**Enzymatic Assay of SUCCINIC THIOKINASE  
(EC 6.2.1.4)**

**PRINCIPLE:**



Abbreviations:

GTP = Guanosine 5'-Triphosphate

GDP = Guanosine 5'-Diphosphate

CoA = Coenzyme A

Pi = Orthophosphate

**CONDITIONS:** T = 30°C, pH = 7.4, A<sub>235nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 50 mM Tris-Succinate Buffer, pH 7.4 at 30°C  
(Prepare 50 ml in deionized water using Trizma Succinate, Prod. No. T-9632. Adjust to pH 7.4 at 30°C with 2 M NaOH.)
- B. 10 mM Guanosine 5'-Triphosphate Solution (GTP)  
(Prepare 1 ml in deionized water using Guanosine 5'-Triphosphate, Sodium Salt, Prod. No. G-8877. **PREPARE FRESH.**)
- C. 3 mM Coenzyme A Solution (CoA)  
(Prepare 1 ml in deionized water using Coenzyme A, Sodium Salt, Prod. No. C-3144. **PREPARE FRESH.**)
- D. 300 mM Magnesium Chloride Solution (MgCl<sub>2</sub>)  
(Prepare 1 ml in deionized water using Magnesium Chloride, Hexahydrate, Prod. No. M-0250.)

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**REAGENTS:** (continued)

E. Succinic Thiokinase Solution  
(Immediately before use, prepare a solution containing 1.25 - 2.5 units/ml of Succinic Thiokinase in cold deionized water.)

**PROCEDURE:**

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

	Test	Blank
Reagent A (Buffer)	2.825	2.825
Reagent B (GTP)	0.025	0.025
Reagent C (CoA)	0.05	0.05
Reagent D (MgCl <sub>2</sub> )	0.10	0.10

Mix by inversion and equilibrate to 30°C. Monitor the A<sub>235nm</sub> until constant, using a suitably thermostatted spectrophotometer. Then add:

Deionized Water	-----	0.010
Reagent E (Enzyme Solution)	0.010	-----

Immediately mix by inversion and record the increase in A<sub>235nm</sub> for approximately 5 minutes. Obtain the r A<sub>235nm</sub>/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/mg enzyme} = \frac{(\text{r } A_{235\text{nm}}/\text{min Test} - \text{r } A_{235\text{nm}}/\text{min Blank})}{(4.0) (\text{mg enzyme/ml RM})}$$

4.0 = Change in the millimolar extinction coefficient of Succinyl-CoA to CoA at 235nm

RM = Reaction Mix

**UNIT DEFINITION:**

One unit will convert 1.0 μmole of succinate to succinyl-CoA per minute at pH 7.4 at 30°C in the presence of coenzyme A and GTP.

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**FINAL ASSAY CONCENTRATION:**

In a 3.01 ml reaction mix, the final concentrations are 47 mM tris succinate, 0.08 mM GTP, 0.05 mM CoA, 10 mM MgCl<sub>2</sub> and 0.0125-0.025 units succinic thiokinase.

**REFERENCE:**

(1964) *J. Biol. Chem.*, **239**, 1961

**NOTES:**

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

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