

**Enzymatic Assay of CROTONASE
(EC 4.2.1.17)**

PRINCIPLE:

Trans-Crotonoyl CoA + H₂O $\xrightarrow{\text{Crotonase}}$ Hydroxybutyryl CoA

Abbreviations:

CoA = Coenzyme A

CONDITIONS: T = 25°C, pH = 7.5, A_{263nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 1000 mM Tris HCl, pH 7.5 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 7.5 with 1.0 N HCl.)
- B. 0.1% w/v Albumin, Chicken Egg
(Prepare 50 ml in deionized water using Albumin, Chicken Egg, Grade V, Prod. No. A-5503.)
- C. 3.2 mM Crotonoyl CoA, Lithium Salt
(Prepare 10 ml in deionized water using Crotonoyl CoA, Lithium, Prod. No. C-6146.)
- D. 100 mM Ethylenediaminetetraacetic Acid, Dipotassium
(Prepare 50 mL in deionized water using Stock No. ED2P.)
- E. Assay Buffer
(Prepare 3 ml using 1 ml of Reagent A, 1 ml of Reagent B, 0.15 ml of Reagent D, and 0.85 ml of deionized water.)
- F. Crotonase Enzyme Solution
(Immediately before use, prepare a solution containing 0.33 units per ml in ice cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent E (Assay Buffer)	0.4	0.4
Deionized Water	2.4	2.4
Reagent C (Crotonoyl CoA, Lithium)	0.1	0.1

Mix by inversion and incubate to 25°C using a suitably thermostatted spectrophotometer. (The absorbance should be between 0.5 and 0.7.) Monitor the A_{263} until constant then add:

Reagent F (Enzyme Solution)	0.1	-----
Deionized water	-----	0.1

Immediately mix by inversion and record the decrease in A_{263} . The decrease in A_{263} should not exceed 0.06 A_{263}/min . Obtain the $r A_{263}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{(r A_{263\text{nm}}/\text{min Test} - r A_{263\text{nm}}/\text{min Blank})}{(6.7) (\text{mg enzyme/ml RM})}$$

6.7 = Millimolar extinction coefficient of Crotonoyl CoA
at 263 nm
RM = Reaction Mix

UNIT DEFINITION:

One unit will hydrate 1.0 μmole of crotonoyl coenzyme A to hydroxybutyryl coenzyme A per minute at 7.5 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3 ml reaction mix, the final concentrations are
44 mM Tris HCl, 0.0044% Albumin, Chicken egg,
0.67 mM EDTA, dipotassium, and 0.107 mM Crotonoyl CoA,
Lithium.

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NOTES:

1. All product 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.