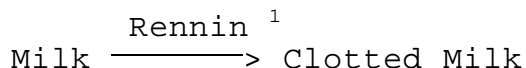


Enzymatic Assay of RENNIN
(EC 3.4.23.4)

PRINCIPLE:



CONDITIONS: T = 30°C

METHOD: Clotting time

REAGENTS:

- A. 1 M Calcium Chloride Solution
(Prepare 5 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)
- B. 10.4% (w/v) Milk Solution with 10 mM Calcium Chloride (Milk)
(Prepare by dissolving 20.9 g of Carnation Instant Nonfat Dry Milk Powder in 200 ml of deionized water or use skimmed cow's milk. Then add 2 ml of Reagent A.)
- C. 0.1% (w/v) Rennet Standard Solution (Rennet Std)
(Prepare 10 ml in deionized water using Rennet, Sigma Prod. No. R-3376.)
- D. Rennin Enzyme Solution (Rennin)
(Immediately before use, prepare a solution containing 0.01 - 0.05 mg/ml of Rennin in cold deionized water. Dilute accordingly so that the clotting time is 0.75 - 1.5 times that of Reagent C.)

**Enzymatic Assay of RENNIN
(EC 3.4.23.4)**

PROCEDURE:

Step 1:

Pipette (in milliliters) the following reagents into a 50 ml Erlenmeyer flask:

	<u>Control</u>
Reagent B (Milk)	10.00

Incubate at 30°C in a water bath for 45 minutes. At t_0 add:

Reagent C (Rennet Std)	1.00
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Swirl gently (Erlenmeyer flask) at 30°C in a water bath. Stop timing and swirling when a white-translucent semi-liquified film appears on the side of the flask above the milk. This is t_1 . After t_1 , the milk will continue to congeal.

Step 2:

Pipette (in milliliters) the following reagents into a suitable test tube.

	<u>Test</u>
Reagent B (Milk)	10.00

Incubate at 30°C in a water bath for 45 minutes. Then add:

Reagent D (Rennin)	1.00
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Swirl gently (Erlenmeyer flask) at 30°C in a water bath. Stop timing and swirling when a white-translucent semi-liquified film appears on the side of the flask above the milk. This is t_2 .

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(t_1)(df)}{(t_2)(1)}$$

t_1 = Clotting time of Rennet
df = Dilution factor

t_2 = Clotting time of Rennin
l = Volume (in milliliter) of enzyme

**Enzymatic Assay of RENNIN
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CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

One unit will coagulate 10 ml of milk per minute at 30°C.

FINAL ASSAY CONCENTRATIONS:

In a 11.00 ml reaction mix, the final concentrations are 9.5% (w/v) milk, 9 mM calcium chloride, and 0.01 - 0.05 mg rennin.

REFERENCE:

Foltmann, B. (1970) *Methods in Enzymology*, XIX, 421-437

NOTES:

1. Rennin clots milk by cleavage of a single Ser-Phe¹⁰⁵+Met-Ala bond in the ?-chain of casein.
2. It is important that the enzyme solution is assayed within 15 minutes of the standard rennet solution using the same substrate preparation incubated for approximately the same time.
3. This assay is based on the cited reference.
4. 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.