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

**PRINCIPLE:**

\[
\text{Rennin}^1 \quad \text{Milk} \quad \rightarrow \quad \text{Clotted Milk}
\]

**CONDITIONS:**  
\[ T = 30^\circ C \]

**METHOD:**  
Clotting time

**REAGENTS:**

A. 1 M Calcium Chloride Solution  
(Prepare 5 ml in deionized water using Calcium Chloride, Dihydrate.)

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.)

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.)
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PROCEDURE:

Step 1:

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

- Control
  - Reagent B (Milk) 10.00

Incubate at 30°C in a water bath for 45 minutes. At t₀ add:

- Reagent C (Rennet Std) 1.00

Swirl gently (Erlenmeyer flask) at 30°C in a water bath. Stop timing and swirlling when a white-translucent semi-liquified film appears on the side of the flask above the milk. This is t₁. After t₁, the milk will continue to congeal.

Step 2:

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

- Test
  - Reagent B (Milk) 10.00

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

- Reagent D (Rennin) 1.00

Swirl gently (Erlenmeyer flask) at 30°C in a water bath. Stop timing and swirlling when a white-translucent semi-liquified film appears on the side of the flask above the milk. This is t₂.

CALCULATIONS:

\[
\frac{(t_1)(df)}{(t_2)(1)}
\]

Units/ml enzyme =

\( t_1 = \text{Clotting time of Rennet} \)

\( df = \text{Dilution factor} \)
\[ t_2 = \text{Clotting time of Rennin} \]
\[ l = \text{Volume (in milliliter) of enzyme} \]
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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:


NOTES:

1. Rennin clots milk by cleavage of a single Ser-Phe^{105}+Met-Ala bond in the \(\beta\)-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.