Suitability Assay for AZOCASEIN as a Substrate for Trypsin

PRINCIPLE:

$$\text{Azocasein} + H_2O \xrightarrow{\text{Trypsin}} \text{Colored Reaction Products}$$

CONDITIONS: T = 37°C, pH 8.3, A_{440nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

A. 0.50% (w/v) Sodium Bicarbonate Buffer ($NaHCO_3$), pH 8.3 at 37°C
(Prepare 100 ml in deionized water using Sodium Bicarbonate, Sigma Prod. No. S-8875. Adjust to pH 8.3 at 37°C with 1 M HCl.)

B. 2.50% (w/v) Azocasein Solution (Azocasein)
(Prepare 15 ml in Reagent A using Azocasein, Sigma Prod. No. A-2765. Gentle heating and stirring may be needed to form a solution.)

C. 5.0% (v/v) Trichloroacetic Acid Solution (TCA)
(Prepare 40 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, 100% w/v, Sigma Stock No. 490-10.)

D. 500 mM Sodium Hydroxide (NaOH) Solution
(Prepare 50 ml in deionized water using Sodium Hydroxide Solution, 1.0 Normal, Sigma Stock No. 930-65.)

E. Trypsin Enzyme Solution (Trypsin)
(Prepare a solution containing 10 mg solid/ml of Trypsin, Sigma Prod. No. T-4665 in Reagent A.)

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PROCEDURE:

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

	Test	Blank
Reagent B (Azocasein)	2.50	2.50
Reagent A (Buffer)	1.50	2.50

Mix by swirling and equilibrate to 37°C. Then add:

Reagent E (Trypsin)	1.00	------

Mix by swirling and incubate at 37°C for exactly 30 minutes.

Remove a 1 ml aliquot from both the Test and Blank solutions and place in a suitable container. Then add:

Reagent C (TCA)	4.0	4.0

Mix by swirling and filter using Whatman #54 paper or 0.45 μm syringe filters. Remove a 1 ml aliquot from both the Test and Blank solutions and place in a suitable container. Then add:

Reagent D (NaOH)	3.0	3.0

Mix by swirling and transfer the Test and Blank solutions to suitable cuvettes. Record the A_{440nm} for both the Test and Blank using a suitable spectrophotometer.

CALCULATION:

$\Delta A_{440nm} = A_{440nm}$ Test - A_{440nm} Blank

Compare the A_{440nm} of the Test to that of a control sample. The absorbance should be similar.

FINAL ASSAY CONCENTRATION:

In a 5.00 ml reaction mix, the final concentrations are 0.50% (w/v) sodium bicarbonate, 1.25% (w/v) azocasein, and 10 mg trypsin.

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REFERENCE:

Tomarelli, R.M. *et al* (1949) *J. Lab. Clin. Med.* **34**, 428

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.