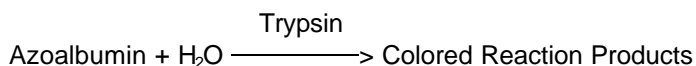


Suitability Assay for AZOALBUMIN as a Substrate for Trypsin

PRINCIPLE:



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

METHOD: Colorimetric

REAGENTS:

- A. 0.50% (w/v) Sodium Bicarbonate Buffer (NaHCO₃), pH 8.3 at 37°C
(Prepare 50 ml in deionized water using Sodium Bicarbonate, Sigma Prod. No. S-6014. Adjust to pH 8.3 at 37°C with 1 M HCl.)
- B. 2.5% (w/v) Azoalbumin Solution (Azoalbumin)
(Prepare 25 ml in Reagent A using Azoalbumin, Sigma Prod. No. A-2382. Gentle heating and stirring may be needed to form a solution. Adjust to pH 8.3 at 37°C, if necessary, with 1 M HCl.)
- C. 5.0% (w/v) Trichloroacetic Acid Solution (TCA)
(Prepare 50 ml in deionized water using Trichloroacetic Acid, 6.1 N Solution, approximately 100% (w/v), Sigma Stock No. 490-10.)
- D. 500 mM Sodium Hydroxide Solution (NaOH)
(Prepare 20 ml in deionized water using Sodium Hydroxide Solution, 1.0 N, Sigma Stock No. 930-65.)
- E. Trypsin Enzyme Solution (Trypsin)
(Immediately before use, prepare a solution containing containing 5 mg solid/ml of Trypsin, Sigma Prod. No. T-8003 in cold Reagent A.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.50	1.50
Reagent B (Azoalbumin)	2.50	2.50

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

Reagent E (Trypsin)	1.00	-----
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Mix by swirling and incubate at 37°C for exactly 10 minutes. Then add:

Reagent E (Trypsin)	-----	1.00
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Immediately remove a 1 ml aliquot from both the Test and Blank solutions and place in a suitable containers. Then add:

Reagent C (TCA)	4.0	4.0
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Mix by swirling and centrifuge solutions for five minutes. Remove a 1 ml aliquot from the supernatants of both the Test and Blank and place in suitable containers. Then add:

Reagent D (NaOH)	3.0	3.0
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Mix by swirling and transfer the Test and Blank solutions to suitable cuvettes. Record the $A_{440\text{nm}}$ for both the Test and Blank in a suitable spectrophotometer.

CALCULATION:

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

Compare the $A_{440\text{nm}}$ 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) azoalbumin, and 5 mg trypsin.

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as a Substrate for Trypsin**

REFERENCE:

Tomarelli, R.M., Charney, M.S, and Harding, M.L. (1949) *Journal of Laboratory and Clinical Medicine*
34, 428-433

NOTES:

1. This assay is based on the cited reference.
2. 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.