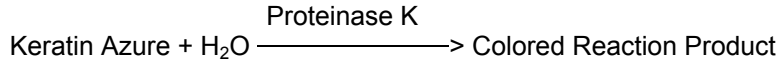


SIGMA QUALITY CONTROL TEST PROCEDURE

Suitability Assay for KERATIN AZURE as a Substrate for Proteinase K

PRINCIPLE:



CONDITIONS: T = 37 °C, pH = 7.5, A_{595nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 50 mM Sodium Phosphate Buffer, pH 7.5 at 37 °C
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876. Adjust to pH 7.5 at 37 °C with 1 M HCl.)
- B. Keratin Azure
(Use Keratin Azure, Sigma Prod. No. K-8500.)
- C. Proteinase K Enzyme Solution
(Immediately before use, prepare a solution containing 100 units/ml of Proteinase K, Sigma Prod. No. P-6556, in cold deionized water.)

PROCEDURE:

Weigh (in milligrams) the following reagents into suitable containers:

	<u>Test 1</u>	<u>Test 2</u>	<u>Test 3</u>	<u>Test 4</u>	<u>Blank</u>
Reagent B (Keratin Azure)	20	20	20	20	20

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

Reagent A (Buffer)	4.00	4.00	4.00	4.00	4.00
Deionized Water	0.40	0.30	0.20	0.10	0.50

Mix by swirling and equilibrate at 37 °C for 10 minutes in a water bath. Then add:

	<u>Test 1</u>	<u>Test 2</u>	<u>Test 3</u>	<u>Test 4</u>	<u>Blank</u>
Reagent C (Proteinase K)	0.10	0.20	0.30	0.40	-----

Mix by swirling and incubate at 37 °C for exactly 1 hour on a metabolic shaker where constant mixing can be maintained.

Centrifuge the solutions for 10 minutes and transfer the supernatants to suitable cuvettes. Record

the A_{595nm} for both the Tests and Blanks with a suitable spectrophotometer.

CALCULATIONS:

$$\Delta A_{595nm} \text{ Test} = A_{595nm} \text{ Test} - A_{595nm} \text{ Blank}$$

$$\Delta A_{595nm} / \text{hour/mg enzyme} = \frac{\Delta A_{595nm} \text{ Test}}{\text{mg enzyme/RM}}$$

RM = Reaction Mixture

SPECIFICATION:

Compare the $\Delta A_{595nm} / \text{hour/mg enzyme}$ of the test to that of a control sample. These values should be similar.

FINAL ASSAY CONCENTRATION:

In a 4.50 ml reaction mix, the final concentrations are 44 mM sodium phosphate, 0.44% (w/v) keratin azure, and 10 - 40 units proteinase K.

REFERENCE:

Wainwright, M. (1982), *Experientia* **38**, 243-244

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 purpose s. For a current copy of Sigma's quality control procedure contact our Technical Service Department.