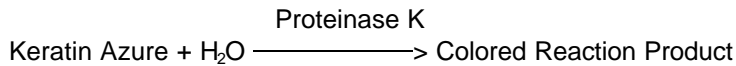


**Suitability Assay for KERATIN AZURE  
as a Substrate for Proteinase K**

**PRINCIPLE:**



**CONDITIONS:** T = 37°C, pH = 7.5, A<sub>595nm</sub>, 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

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**PROCEDURE:** (continued)

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 B (Keratin Azure)	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 purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**