



Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE

Suitability Assay for AZO DYE IMPREGNATED COLLAGEN

PRINCIPLE:

Azocoll + H₂O $\xrightarrow{\text{Protease}}$ Colored Reaction Products

Abbreviation used:

Azocoll = Azo Dye Impregnated Collagen

CONDITIONS: T = 37°C, pH = 7.0, A_{520nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 100 mM Potassium Phosphate Buffer, pH 7.0 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Sigma Prod. No. P-5379. Adjust to pH 7.8 at 37°C with 1 M KOH.)
- B. Azo Dye Impregnated Collagen (Azocoll)
(Use Azo Dye Impregnated Collagen, Sigma Prod. No. A-4341.)
- C. Protease Enzyme Solution (Protease) Step 1
(Immediately before use, prepare a solution containing 0.09 - 0.13 unit/ml of Protease, Sigma Prod. No. P-5380, in cold Reagent A. Store at 0 - 4°C.)
- D. Protease Enzyme Solution (Protease) Step 2
(Immediately before use, prepare a solution containing 10.0 - 12.00 units/ml of Protease, Sigma Prod. No. P-5380, in cold Reagent A. Store at 0 - 4°C.)

PROCEDURE:

Weigh (in milligrams) the following reagents into 4 dram vials:

	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Blank
Reagent B (Azocoll)	25	25	25	25	25	25	25

Then add (in milliliters):

Reagent A (Buffer)	5.08	5.06	5.04	5.02	5.00	5.00	5.00
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PROCEDURE: (continued)

Equilibrate to 37°C. Then add at 1 minute time intervals:

	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Blank
Reagent C (Protease)	0.02	0.04	0.06	0.08	0.10	-----	-----
Reagent D (Protease)	-----	-----	-----	-----	-----	0.10	-----

Incubate at 37°C for exactly 15 minutes in a shaking water bath (60 cycles/minute).

Remove the vials and immediately filter the suspension through 0.45 micron syringe filters. Transfer the filtered solutions to suitable cuvettes and record the $A_{520\text{nm}}$ using a suitable spectrophotometer.

CALCULATIONS:

Sample Curve:

$$\Delta A_{520\text{nm}} \text{ Sample} = A_{520\text{nm}} \text{ Test} - A_{520\text{nm}} \text{ Test Blank}$$

Plot the $\Delta A_{520\text{nm}}$ Sample vs units of protease per assay.

SPECIFICATIONS:

The sample is suitable if it has similar results compared to that of a control. The plot of absorbance versus units of protease should be nonlinear. The $\Delta A_{520\text{nm}}$ of Test 6 >1.0 .

FINAL ASSAY CONCENTRATION:

In a 5.00 ml reaction mix, the final concentrations are 100 mM phosphate, 0.5% (w/v) azo dye impregnated collagen, and 0.009 - 0.013 unit protease.

REFERENCE:

Chavira, R. Jr., Burnett, T.J., and Hageman, J.H. (1984) *Analytical Biochemistry* **136**, 446-450

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NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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