



Chitosan Colorimetric Assay Kit

(Catalog # K995-100; 100 assays, Store at RT)

I. Introduction:

Chitosan is a linear cationic amino-polysaccharide of D-glucosamine and N-acetylglucosamine. It can be extracted by the deacetylation of Chitin from the shells of marine crustaceans including shrimps, lobsters, crabs etc. Chitosan is abundant, non-toxic, biodegradable and is biocompatible. It has a wide range of applications in industries such as preservatives in food industry, as antimicrobials in agricultural industry, as DNA/siRNA nanoparticles in biotechnology, as hydrogels in biomedical science, for drug delivery in pharmaceutical industry etc. Additionally, small oligomers of Chitosan are studied for their anti-oxidant, anti-inflammatory, immune enhancing, anti-tumor, antibacterial properties. BioVision's Chitosan Assay Kit is the first commerically available kit to measure the Chitosan content in various samples. In this assay, Chitosan is converted to an intermediate to generate a pink colored product, which is then detected by absorbance at 532 nm. The absorbance signal is directly proportional to the Chitosan concentration. BioVision's Chitosan Assay Kit is rapid, sensitive and convenient. It can detect as low as 10 µg/ml under assay conditions.

Converter Detector

Chitosan — Intermediate — Pink Product (Measured at O.D. 532 nm)

II. Application:

• Determination of Chitosan in different solid and liquid samples.

III. Sample Type:

- · Foods (breads, meats, pet foods and drug capsules)
- Liquid samples (serum, plasma, water and urine)
- Crops (plant and fruit surface)

IV. Kit Contents:

Components	K995-100	Cap Code	Part Number
Chitosan Assay Solution	110 ml	WM	K995-100-1
Chitosan Standard	1 Bottle	Red	K995-100-2
Chitosan Converter	1 Bottle	Blue	K995-100-3
Chitosan Detector	1 Bottle	NM	K995-100-4

V. User Supplied Reagents and Equipment:

- dH₂O
- Eppendorf tube
- 96-well clear flat-bottom plate
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store the kit at RT. The kit components are stable for one year when stored as recommended. Read the entire protocol before performing the experiment.

- Chitosan Assay Solution: Ready to use. Store at room temperature (RT).
- Chitosan Standard: Weigh out 5 mg of Chitosan and vortex it with 0.5 ml of Chitosan Assay Solution for 5 min to prepare a 10 mg/ml Chitosan Standard stock solution. Store at 4°C. The reconstituted Chitosan Standard stock solution is stable for 3 weeks.
- Chitosan Converter: For 10 assays, weigh out 17 mg of Chitosan Converter. Vortex it with 0.5 ml of dH₂O to prepare Chitosan Converter solution. Store at 4°C. Use within 2 weeks.
- Chitosan Detector: For 10 assays, weigh out 15 mg of Chitosan Detector. Vortex it with 2.5 ml of dH₂O to prepare Chitosan Detector solution. Store at 4°C. Use within 2 weeks. Note: If precipitate is observed, warm the Chitosan Detector solution in a 55°C water bath to dissolve the precipitate before use.

VII. Chitosan Assay Protocol:

1. Sample Preparation: For foods samples (breads, meats, pet foods and drug capsules): Weigh 50 mg of the Sample. If possible, cut the Samples into small pieces. Add 1 ml of the Chitosan Assay Solution to the Sample(s) and homogenize it for 10 min using a pestle. Let it incubate at RT for 10 min. Centrifuge the Sample at 12,000 x g and RT for 10 min and collect the supernatant. Add 200 µl of the supernatant into an eppendorf tube for the assay.

For serum or urine samples: Dilute the Sample 20 fold by adding 25 µl of the Sample to 475 µl of Chitosan Assay Solution. Add 200 µl of the diluted Sample into an eppendorf tube for the assay. For other liquid samples, dilute the Sample 2-10 fold using the Chitosan Assay Solution. Add 200 µl of the diluted Sample into an eppendorf tube for assay.

For crop samples (plant and fruit surface): Wet a cotton swab with Chitosan Assay Solution and gently rub the surface of the tested Sample. Then stir the cotton swab in an eppendorf tube containing 1 ml of the Chitosan Assay Solution for 2 min. Add 200 µl of the diluted Sample into an eppendorf tube for the assay.

2. Chitosan Standard Curve Preparation: Prepare a 100 μg/ml Chitosan Standard solution by adding 20 μl of 10 mg/ml Chitosan Standard stock solution to 1980 μl of Chitosan Assay Solution. Add 0, 40, 80, 120, 160 and 200 μl of the 100 μg/ml Chitosan Standard Solution into different eppendorf tubes to generate 0, 20, 40, 60, 80 and 100 μg/ml of Chitosan Standard per tube respectively. Adjust the final volume to 200 μl per tube using Chitosan Assay Solution.

6/19





LB

LB +

Spike

100

0

Serum

Serum +

Spike

- 3. Color Development: Add 5 µl of Chitosan Converter Solution to each Sample(s). Heat the tubes at 85-90°C for 30 min. After 30 min, add 200 µl of the Chitosan Detector Solution to each tube and heat the tubes at 85-90°C for another 20 min. After 20 min, transfer 250 µl of the Sample to the desired well(s) in a 96-well clear flat-bottom plate.
- 4. Measurement: Measure the O.D. at 532 nm at 25°C immediately.
- 5. Calculation: Subtract 0 Standard reading from all Sample(s) and Standard readings. Plot the Chitosan Standard Curve. Apply the Sample readings to the Chitosan Standard Curve to get A µg/ml of Chitosan in Sample(s).

Chitosan concentration (µq/ml) in Sample(s) = A x D



200

100

0



Figures. A. Chitosan Standard Curve. B. Chitosan detection in shrimp shell and leaf sprayed with Chitosan. C. Spiking experiment. Serum and LB medium are spiked with 400 and 100 µg/ml of Chitosan respectively. Data shows >90% recovery in the assay kit condition.

Leaf (No

Spray)

Leaf

(Sprayed

with

chitosan)

AGE-BSA Shrimp Shell Control

VIII. Related Products:

0.2

0.1

0.0

0

20

40

Chitosan (ug/ml)

60

80

100

Chitotriosidase Activity Assay Kit (Fluorometric) (K512) Acidic Mammalian Chitinase Activity Kit (Fluorometric) (K513) Acidic Mammalian Chitinase Inhibitor Screening Kit (Fluorometric) (K693) EZClick™ O-GlcNAc Modified Glycoprotein Assay Kit (FACS/Microscopy, Green Fluorescence) (K714) Human CellExp[™] CHI3L3, Mouse Recombinant (P1312) Hyaluronic Acid (HA) ELISA Kit (E4626)

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