Product Manual

Chitosan Assay Kit

Catalog Number

XAN-5126 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Chitosan is a linear polysaccharide made up of β-(1→4)-linked D-glucosamine and *N*-acetyl-D-glucosamine. It is made by the process of deacetylation, treating the chitin shells of shrimp and other crustaceans with sodium hydroxide.

Chitosan has been studied for a number of useful applications. It can be used agriculturally as a seed to block fungal infections in plants. Chitosan can be used to help prevent the spoiling of wine. It is used as a self-repairing polyurethane paint coating. In medicine, Chitosan reduces bleeding in bandages and can act as an antibacterial agent. Additionally, Chitosan has been used in drug delivery systems for treatment of anxiety, migraines, glaucoma, lung cancer, and diabetes therapy. Quantum dots decorated with Chitosan have been used in bioimaging to diagnose cancer. Derivatives of Chitosan have been shown to have antioxidant properties as well as enhance immune responses against pathogenic microorganisms.

Cell Biolabs' Chitosan Assay Kit provides a convenient colorimetric method for the detection of Chitosan from food, animal, or plant samples. First, Chitosan in the unknown samples or standards is converted to a detectable intermediate by Assay Reagent A. Assay Reagent B is added to form a colorimetric product. The final solution is transferred to a 96 well plate and measured by a plate spectrophotometer. The amount of Chitosan in the unknown samples is determined by comparing with a predetermined Chitosan standard curve. The provided reagents are sufficient for the evaluation of 96 assays including standards and unknown samples. The kit is sensitive enough to detect 6.25 µg/mL Chitosan.

Related Products

1. XAN-5040: OxiSelect™ Trolox Equivalent Antioxidant Capacity (TEAC) Assay Kit
2. XAN-5077: OxiSelect™ Flavanoid Assay Kit
3. XAN-5084: OxiSelect™ Free Hydrogen Sulfide Assay Kit

Kit Components (shipped at room temperature)

1. Chitosan Standard (Part No. 51261B): One 500 µL vial containing 4 mg/mL Chitosan.
2. Assay Reagent A (Part No. 51262B): One 500 µL vial.
3. Thiobarbituric Acid (TBA) (Part No. 51265A): One 200 milligram tube.
4. 10X Assay Buffer (Part No. 51264A): One 10 mL bottle of 10% Acetic Acid.

Materials Not Supplied

1. Distilled water
2. 96 well ELISA strips or 96 well microtiter plate
3. 0.6 mL or 1.5 mL microcentrifuge tubes

4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 540 nm
8. 2 N HCl
9. 2 N NaOH
10. Solid NaOH to make 12.5 N NaOH
11. Mortar and pestle
12. Vacuum drying apparatus, oven, or heat block

Storage

Upon receipt, store Thiobarbaturic Acid (TBA) at room temperature. Store all other components at 4°C.

Preparation of Standard Curve

Prepare a dilution series of Chitosan standards in the concentration range of 0 to 500 µg/mL by diluting the Collagen Standard in 1X Assay Buffer (Table 1).

Standard Tubes	4 mg/mL Chitosan Standard (µL)	1X Assay Buffer (µL)	Chitosan (µg/mL)
1	100	900	400
2	500 of Tube #1	500	200
3	500 of Tube #2	500	100
4	500 of Tube #3	500	50
5	500 of Tube #4	500	25
6	500 of Tube #5	500	12.5
7	500 of Tube #6	500	6.25
8	0	500	0

Table 1. Preparation of Chitosan Standards.

Preparation of Reagents

- 1X Assay Buffer: Dilute the 10X Assay Buffer to 1X with deionized water. Stir to homogeneity.
- Assay Reagent B: Prepare the TBA Reagent just before use. Prepare a 5.8 mg/mL solution of Assay Reagent B by weighing out an amount of TBA needed for all samples and standards (e.g. 23 mg of TBA is enough to prepare 20 tests). Add distilled water to the TBA and warm the mixture at 60°C for 45-60 minutes. Mix well every 20 minutes to resuspend all the TBA.

Note: Assay Reagent B is stable for 24 hours. Do not store or reuse diluted solutions. Prepare only enough for the current experiment

Preparation of Samples

The following recommendations are only guidelines for extraction of Chitosan from shrimp, lobster, or crab shells, and may be altered to optimize or complement the user's experimental design.

- Shrimp, Lobster, or Crab Shells:

1. Wash 0.5 to 5 grams of shell waste with distilled water and then incubate under vacuum, in a conical tube on a heat block, or in an oven for several hours at 80-100°C until visually dry. Thoroughly grind the dried shells to a powder using a mortar and pestle.
2. Demineralize the powder by slowly adding 15 mL of 2 N HCl per gram of shells and stirring or mixing for 2 hours at room temperature. Pellet the demineralized powder at 20000 xg for 10 minutes.
3. Wash with 40 mL of distilled water. Monitor the pH of the supernatant with pH paper and repeat wash steps until pH reaches 5 (usually 5 to 6 washes). Dry the powder on a heat block or in an oven for several hours at 80-100°C until visually dry.
4. Deproteinate the powder by adding 20 mL of 2 N NaOH per gram of powder and stirring or mixing for 2 hours at room temperature.
5. Repeat step 3 above.
6. Treat the dried powder with 5 mL of 12.5 N NaOH per gram of powder and incubate overnight at 95°C in a sealed container.
7. Repeat step 3 above to produce dried Chitosan powder.
8. Weigh out 10 to 50 mg of the extracted Chitosan powder and resuspend in 1% Acetic Acid at 1 mg/mL, and dilute as necessary in 1% Acetic Acid.

- Serum, Plasma or Urine: Dilute at least 2-fold into 1% Acetic Acid.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, unknown and standard should be assayed in duplicate.
2. Add 200 µL of Chitosan standards or unknown samples to a microcentrifuge tube.
3. Add 5 µL of Assay Reagent A to each tube and incubate 30 minutes at 85°C.
4. Add 200 µL of Assay Reagent B to each tube and incubate 20 minutes at 85°C.
5. Transfer 250 µL of the sample to a 96-well microplate.
6. Read absorbance of each well on a microplate reader using 540 nm as the primary wavelength.

Example of Results

The following figures demonstrate typical Chitosan Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

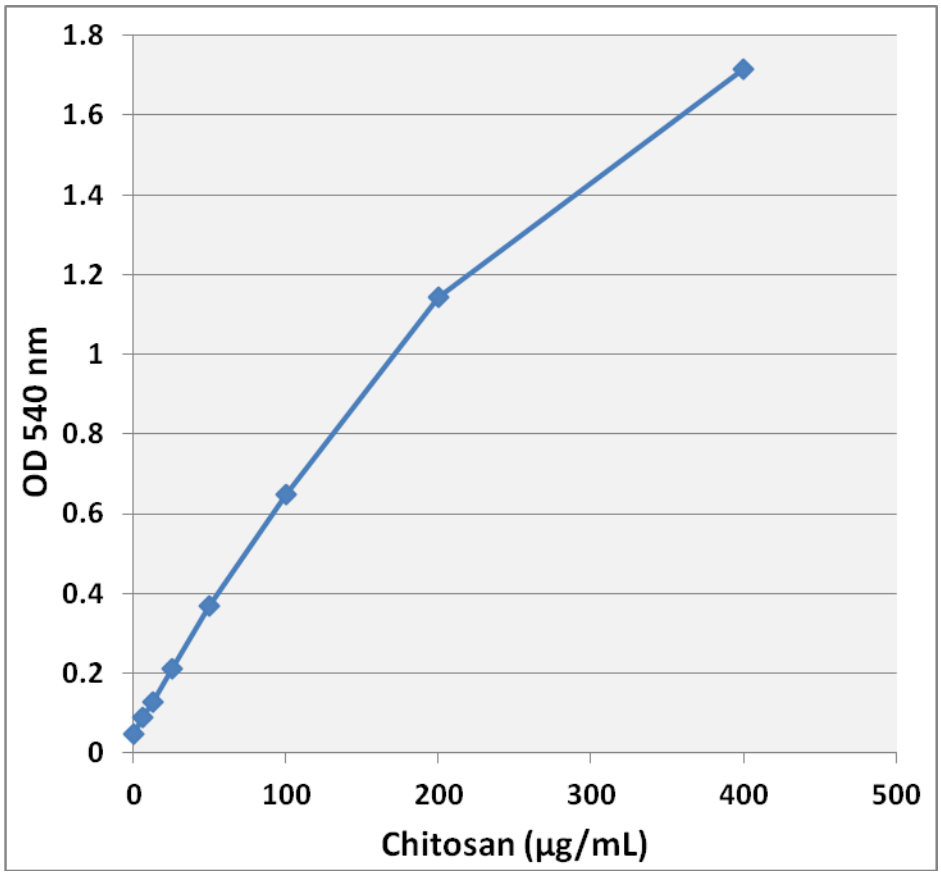

Figure 1: Chitosan Standard Curve.

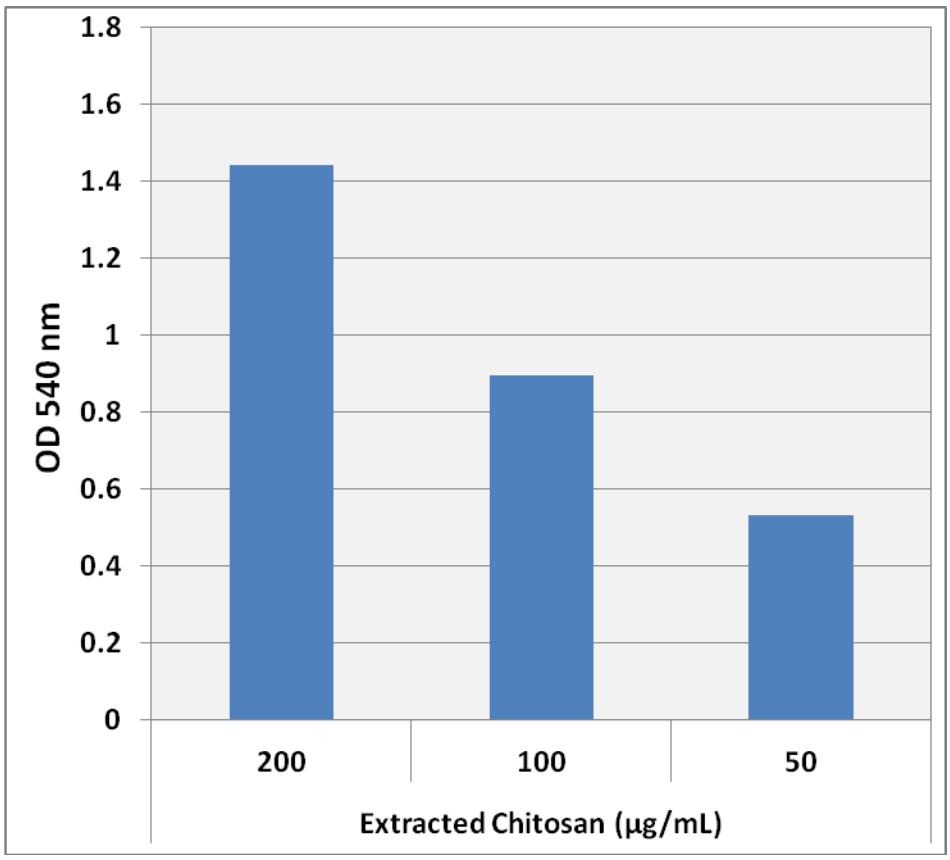

Figure 2: Detection of Chitosan in Shrimp Shells. Chitosan was extracted from shrimp shells according to the Preparation of Samples Section. Samples were tested according to the Assay Protocol.

References

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