Chitosan Assay kit

ARG83387 Chitosan Assay kit is an assay kit for Chitosan in Serum, plasma, urine, and shell.

Catalog number: ARG83387

Package: 100 assay

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INTRODUCTION

Chitosan is a linear polysaccharide composed of randomly distributed β-linked D-glucosamine and N-acetyl-D-glucosamine. It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance, such as sodium hydroxide.

Chitosan has a number of commercial and possible biomedical uses. It can be used in agriculture as a seed treatment and biopesticide, helping plants to fight off fungal infections. In winemaking, it can be used as a fining agent, also helping to prevent spoilage. In industry, it can be used in a self-healing polyurethane paint coating. In medicine, it is useful in bandages to reduce bleeding and as an antibacterial agent; it can also be used to help deliver drugs through the skin.

PRINCIPLE OF THE ASSAY

ARG83387 Chitosan Assay Kit measures Chitosan content in biological samples. Chitosan in the unknown samples or standards is converted to a detectable intermediate by Reagent A. Reagent B is added to form a colorimetric product. Samples are compared to a known concentration of Chitosan standard. The intensity of the color is measured at a wavelength of 540 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Reagent B at RT.

Store other component at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
Standard (4 mg/mL)	500 µL	4°C
10X Assay Buffer	10 mL	4°C
Reagent A	500 µL	4°C
Reagent B	20 mL	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540 nm
- Flat bottomed 96-well microplate and tube.
- 2N HCl, 2N NaOH, 12.5 N NaOH and deionized water
- Pipettes and pipette tips

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection.
- Upon received, store Reagent B at RT. Store other component at 4°C. Use the kit before expiration date and avoid freeze / thaws.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to 4°C / room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Urine - Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months.

Shell- Wash 0.5 to 5 grams of shell with distilled water and then incubate under vacuum, in a conical tube on a heat block or oven until dry. Grind the dried shells to a powder using a mortar and pestle.

REAGENT PREPARATION

- 1X Assay Buffer: Dilute the 10X Assay Buffer with deionized water to yield 1X Assay Buffer. Store at RT.
- Serum, Plasma or Urine sample: Dilute at least 2 fold into 1% Acetic Acid
- Shell Sample:

1. Demineralize the powder by adding 15 mL 2 N HCl per gram of shells and stirring or mixing for 2 hours at room temperature. Pellet the demineralized powder at 20,000g for 10 minutes.
2. Wash with 40 mL distilled water until pH 5.0 (usually 5 to 6 washes). Dry the powder in a conical tube on a heat block or oven until dry..
3. 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.
4. Wash as step 2.
5. 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.
6. Wash as step 2 to produce dried Chitosan powder.
7. 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.

- Standards: Add 100 µl of 4 mg/mL stock standard into 900 µl 1X Assay Buffer to generate a standard with 400 µg/mL of Chitosan. Dilute the standards with 1X Assay Buffer serves as zero standard (blank standard, 0 µg/mL). The example of the standards dilution table is as below:

Standard	Chitosan (µg/mL)	Volume of 1X Assay Buffer (µL)	Volume of Chitosan (µL)
S1	400	900	100 (4 mg/mL stock)
S2	200	500	500(S1)
S3	100	500	500(S2)
S4	50	500	500(S3)
S5	25	500	500(S4)
S6	12.5	500	500(S5)
S7	6.25	500	500(S6)
S8	0	500	0

ASSAY PROCEDURE

Each samples should be assayed in at least duplicates, one to be treated with PPDK and one without, to measure endogenous background.

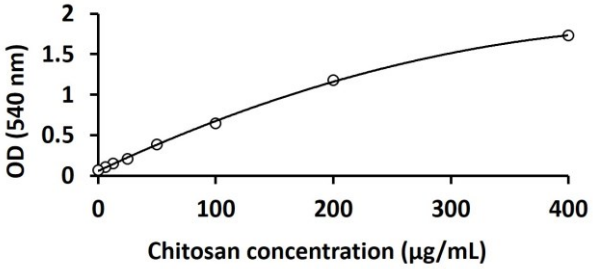
1. Add **200 µl** of **standard** and **sample** to tube.
2. Add **5 µL** of **Reagent A** to each tube, Incubate for **30 minutes** at 85°C
3. Add **200 µL** of **Reagent B** to each tube, Incubate for **20 minutes** at 85°C
4. Transfer **250 µL** of the sample to 96-well microplate.
5. Read O.D. with a microplate reader at **540 nm** immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of standards and samples.
2. Subtract the average value of Standard 0 from all standard value.
3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

EXAMPLE OF TYPICAL STANDARD CURVE

The following table shows the OD readings of a run of this assay kit with serial diluted standards



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Chitosan ranged from 6.25-400 µg/mL. The mean MDD was 4.5 µg/mL.