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ab273338
Chitosan Assay Kit (Colorimetric)

For the determination of Chitosan activity in food, crops, serum, plasma, water and urine.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Chitosan Assay Kit (Colorimetric) (ab273338) is a rapid, sensitive and convenient kit to measure Chitosan content in various samples. For example, foods (breads, meats, pet foods and drug capsules), liquids (serum, plasma, water and urine) and crops (plant and fruit surface).

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.

This kit can detect as low as 10 µg/ml under assay conditions.

2. Protocol Summary

Prepare all reagents and samples.

Prepare standards.

Add all samples to the appropriate wells.

Add Color Developer to the appropriate wells.

Measure the OD at 532 nm at 25°C immediately.

Determine Chitosan concentration using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at RT upon receipt. The kit components are stable for one year when stored as recommended.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
Chitosan Assay Solution	110 ml	RT
Chitosan Converter	1 vial	RT
Developer Mix R	250 mg	RT
Chitosan Standard	1 vial	RT

PLEASE NOTE: Developer Mix R was previously labelled as Developer VII and Chitosan Detector. The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well spectrophotometer
- Eppendorf tubes
- 96-well clear plate with flat bottom
- dH_2O

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

– Briefly centrifuge small vials at low speed prior to opening.

9.1 **Chitosan Assay Solution:**

Ready to use as supplied. Store at room temperature.

9.2 **Chitosan Converter:**

For 10 assays, weigh out 17 mg of Chitosan Converter. Vortex it with 0.5 ml of dH_2O to prepare Chitosan Converter solution. Store at 4°C. Use within 2 weeks.

9.3 **Developer Mix R:**

For 10 assays, weigh out 15 mg of Developer Mix R. Vortex it with 2.5 ml of dH_2O to prepare Developer Mix R solution. Store at 4°C. Use within 2 weeks.

Δ Note: If precipitate is observed, warm the Developer Mix R solution in a 55°C water bath to dissolve the precipitate before use.

9.4 **Chitosan Standard:**

Weigh out 5 mg of Chitosan and vortex it with 0.5 ml of Chitosan Assay Solution for 5 mins 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.

10. Sample Preparation

Foods samples (breads, meats, pet foods and drug capsules):

10.1 Weigh 50 mg of the Sample.
10.2 If possible, cut the Samples into small pieces.
10.3 Add 1 ml of the Chitosan Assay Solution to the Sample(s) and homogenize it for 10 mins using a pestle.
10.4 Let it incubate at RT for 10 mins.
10.5 Centrifuge the Sample at 12,000 x *g* and RT for 10 mins and collect the supernatant.
10.6 Add 200 µl of the supernatant into an Eppendorf tube for the assay.

Serum or urine samples:

10.7 Dilute the Sample 20 fold by adding 25 µl of the Sample to 475 µl of Chitosan Assay Solution.
10.8 Add 200 µl of the diluted Sample into an Eppendorf tube for the assay.
10.9 For other liquid samples, dilute the Sample 2-10 fold using the Chitosan Assay Solution.
10.10 Add 200 µl of the diluted Sample into an Eppendorf tube for assay.

Crop samples (plant and fruit surface):

10.11 Wet a cotton swab with Chitosan Assay Solution and gently rub the surface of the tested Sample.
10.12 Then stir the cotton swab in an Eppendorf tube containing 1 ml of the Chitosan Assay Solution for 2 mins.
10.13 Add 200 µl of the diluted Sample into an Eppendorf tube for the assay.

11. Standard Curve

11.1 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.

11.2 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.

11.3 Adjust the final volume to 200 µl per tube using Chitosan Assay Solution.

Standard #	100 µg/ml Chitosan Standard (µL)	Chitosan Assay Solution (µL)	Chitosan (µg/ml)
1	0	200	0
2	40	160	20
3	80	120	40
4	120	80	60
5	160	40	80
6	200	0	100

12. Assay Procedure

Color Development:

12.1 Add 5 µl of Chitosan Converter Solution to all sample and standard tubes.

12.2 Heat the tubes at 85-90°C for 30 mins.

12.3 After 30 mins, add 200 µl of the Developer Mix R Solution to all sample and standard tubes and heat the tubes at 85-90°C for another 20 mins.

12.4 After 20 mins, transfer 250 µl of the solution to the desired well(s) in a 96-well clear flat-bottom plate.

12.5 Measure the OD at 532 nm at 25°C immediately.

13. Calculations

13.1 Subtract 0 Standard reading from all standard readings.
13.2 Plot the Chitosan Standard Curve.
13.3 Apply the Sample readings to the Chitosan Standard Curve to get A µg/ml of Chitosan in Sample(s).

$$[Chitosan] = A\ x\ D = \ \mu g/mg$$

A = Chitosan amount from the Standard Curve (µg/ml)
D = Sample dilution factor (D=1 for undiluted samples)

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

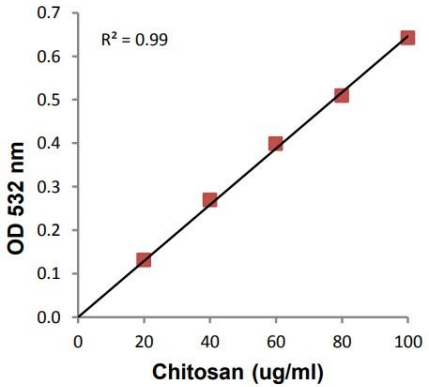

Figure 1. Chitosan Standard Curve.

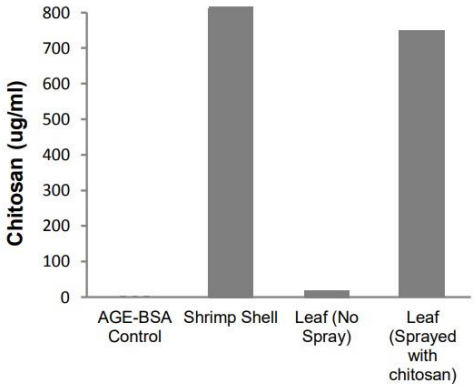

Figure 2. Chitosan detection in shrimp shell and leaf sprayed with Chitosan.

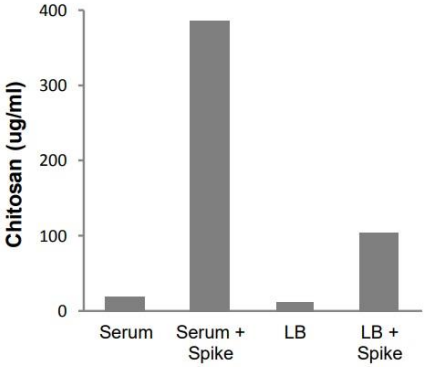

Figure 3. Spiking experiment. Serum and LB medium are spiked with 400 and 100 µg/ml of Chitosan respectively. Data shows >90% recovery in the assay kit condition.

15. FAQ / Troubleshooting

General troubleshooting points are found at https://www.abcam.com/en-us/products/biochemical-assays.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)