Benzoic Acid Detection Assay Kit for Shrimp

Catalog Number: EZ2013-02

For Research Use Only. Not for use in Diagnostic Procedures.

1. Introduction

Benzoic Acid is a white solid that is an extensively used preservative. Although this preservative prevents or delays nutritional losses due to microbiological, enzymatic, or chemical changes of foods during its shelf life there is a suspicion that small amounts of benzene may be formed from benzoic acid in nonalcoholic beverages in the presence of ascorbic acid. Benzoic acid and ascorbic acid are food additives which must be declared on the food. Benzoic acid or E210 is a preservative which also occurs naturally, for instance, in cranberries.

- Highly Sensitive Assay to Screen for Benzoic Acid
- Detection range of 10ppm to 1500ppm.
- Highly reproducibility
- High recovery (>90%) and rapid extraction methods

The kit provides a rapid, simple, sensitive, and reliable test suitable for screening of Benzoic Acid concentration.

2. Contents (96 determinations)

Component Name	Volumes	Storage
Reaction Facilitator	200 µL	-15 to -25°C
Chromophore	1.5 ml x 2	2 - 8°C
Reaction Buffer 1	18 mL	RT
Reaction Buffer 2	2.6 mL	RT
Substrate [S]	700 µL	2 - 8°C
Benzoic Acid Standards (0ppm, 50ppm, 100ppm, 200ppm, 500ppm, 1500ppm)	0.5 mL x 6	RT
Benzoic Acid Spike Solution (15,000ppm)	0.5 mL	RT
96 well plate	1 each	RT

Required materials not included in kit

- Microplate reader (520nm)
- Vortex mixer
- Tissue homogenizer
- 10, 20, 100 and 1000ul pipettes
- 100% methanol

3. Benzoic Acid Test Method

Benzoic Acid is a target in the agricultural industry because of its role as a preservative. The AttoTector Benzoic Acid detection kit is designed specifically to screen for Benzoic Acid in samples. The ability to detect Benzoic Acid in a range from 50 to 1,500 ppm is simple and sensitive as the reaction uses a chromophore that can be detected by eye or can be read in the plate reader at 520nm. In the presence of Benzoic Acid, the rate of chromophore production is reduced in a concentration dependent fashion. The higher the concentration of Benzoic Acid the less color is produced, the color card enables for qualitative determination of concentration.

Attogene test uses the property of Benzoic Acid to inhibit the formation of the chromophore to form a pink color when performed in a sample.

The Benzoic Acid concentration can be measured by reading the absorbance of the reactions at 520nm, generating a standard curve using the standards supplied in the kit and quantifying unknown sample concentrations using linear regression analysis

Measuring range / color- Number of scale graduation	Number of determinations
50 – 100 – 200 – 500 – 1,500 ppm Benzoic Acid	96

4. Instructions

Note: Perform the reaction by mixing the following components in the specific order described below into one well of the provided 96 well plate for each sample, positive and negative control (use a new pipet for each step and for each well).

- Step 1. 167µL of Reaction Buffer 1
- Step 2. 31µL of Chromophore
- Step 3. 2µL of Reaction Facilitator
- Step 4. 25µL of Reaction Buffer 2
- Step 5. 40µL of sample or standards
- Step 6. 7µL of Substrate [S]
- Step 7. Mix the components in the tube by pipetting up and down 3-4 times
- Step 8. Incubate at room temperature for 25 minutes
- Step 9. Read the plate by measuring absorbance at 520nm.

Component	Volume per well
Reaction Buffer 1	167µL
Chromophore	31µL
Reaction Facilitator	2µL
Reaction Buffer 2	25µL
Sample	40µL
Substrate	7µL

5. Master Mix Method

Using a master mix is an acceptable approach to performing the preparation of reagents (ensure overage of 10% to account for pipetting efficiency.

For example, to make a master mix for 15 reactions perform the following in a tube:

- 2.505mL of Reaction Buffer 1
- 465µL of Chromophore
- 30µL of Reaction Facilitator
- 375ul of Reaction Buffer 2
- Mix well

Set up reaction in 96 well plate from the master mix by:

- Step 1. Aliquoting 225µL of the master mix into each well of the 96 well plate.
- Step 2. Add 40µL of sample or standard
- Step 3. Add 7µL of Substrate [S]
- Step 4. Mix the components in the tube by pipetting up and down 3-4 times
- Step 5. Incubate at room temperature for 25 minutes.
- Step 6. Read the plate by measuring absorbance at 520nm.

6. Method Control

It is best to run standards with each unknown sample set to ensure comparable readings from the day, time, and user. If quantitative results are required, make sure to perform duplicate a series of standard curve reactions which can be used to extrapolate the concentration in the sample being analyzed, loading into a 96 well plate and reading the samples at 520nm.

A 15,000 ppm Benzoic Acid Solution is included in the kit can be used to produce sample spiked controls or a set of standards in a negative methanolic extract as needed.

7. Sample Preparation:

Shrimp meat:

- Shrimp should be cut into small pieces with a sharp knife
- The pieces should then be homogenized using a Smart Stick (or similar) handheld blender.
- Add 1 gram of homogenized shrimp into a 15mL tube
- Add 2 mL of 100% methanol
- Vortex for 1 minute
- Centrifuge the sample to pellet debris (3000xg for 20 minutes)
- Transfer the supernatant to a clean tube
- Use 40µL of the sample for evaluation in the test

NOTE: Dilution Factor: 3

Feed:

- Add 1 gram of feed into a 15mL tube
- Add 2 mL of 100% methanol
- Vortext for 1 minute
- Centrifuge the sample to pellet debris (3000xg for 20 minutes)
- Transfer the supernatant to a clean tube
- Use 40µL of the sample for evaluation in the test

NOTE: Dilution Factor: 3

If the concentration of Benzoic acid is not with the range of the curve, additional sample dilutions may be needed to ensure the readings are within range of the standards.

Milk:

- Add 1mL of milk into a 15mL tube
- Add 2mL of 100% methanol
- Vortext for 1 minute
- Centrifuge the sample to pellet debris (3000xg for 20 minutes)
- Transfer the supernatant to a clean tube
- Use 40µL of the sample for evaluation in the test

NOTE: Dilution Factor: 3

Concentration Calculation:

Standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ppm on a logarithmic curve.

Relative absorbance (%) = absorbance standard (or sample) x 100 absorbance zero standard

Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ppm from the standard curve.

The following figure is a typical Benzoic Acid standard curve. The sample detection and quantification limit for this kit are calculated as below.

- Sample detection limit = (50ppm) x (dilution factor)
- Sample quantification limit = (100ppm) x (dilution factor)

Example of standard curve:

Well Contents	OD at 520nm	Bo% (relative absorbance %
0ppm	0.858	100
50ppm	0.687	80%
100ppm	0.572	67%
200ppm	0.401	47%
500ppm	0.197	23%
1,500ppm	0.135	16%

Who we are:

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

Our mission is to:

- Enhance detection technologies
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