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User's Manual

Benzoic Acid Detection Assay Kit for Beverages

REF **DEIANJ42-1**

Σ **96T**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Benzoic Acid Assay Kit is for the quantitative analysis of Benzoic Acid in food.

General Description

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 is a preservative which also occurs naturally, for instance, in cranberries.

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

The kit provides a rapid, simple, sensitive, and reliable test suitable for screening of Benzoic Acid concentration in white wine and other alcoholic beverages.

Principles of Testing

Benzoic Acid is a target in the agricultural industry because of its role as a preservative. The 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.

CD 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:

50 – 100 – 200 – 500 – 1500 ppm Benzoic Acid

Storage

2-8°C

Assay Procedure

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 well 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.

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

375µL 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 well 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.

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.

Sample Preparation:

White wine and other alcoholic beverages:

Add 40µL of sample into the reaction as indicated in the instructions. For highly colored beverages such as red wine, it may be necessary to perform decoloring.

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.

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.

1. Sample detection limit = (50ppm) x (dilution factor)
2. Sample quantification limit = (100ppm) x (dilution factor)