



# Benzoic Acid Detection Assay Kit

## Catalog Number: EZ2013

### **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 E 210 is a preservative which also occurs naturally, for instance, in cranberries. A maximum amount of 150 mg/l benzoic acid may be added to non-alcoholic flavored beverages.

- Highly Sensitive Assay to Screen for Benzoic Acid-
- Visual Readout (red/pink)
- Compatible with the Nix Sensor or plate readers to obtain quantitative results.
- Detection range of 1ppm to 300ppm.

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

### **Kit Contents:**

Component	Amount	Storage Condition
Reaction Facilitator	300 $\mu$ L	2 - 8°C
Chromophore	2 x 1.5 ml	2 - 8°C
0.15% (1,500 ppm Benzoic Acid)	50 $\mu$ L	RT
Substrate (S)	700 $\mu$ L	2 - 8°C
1.5 mL tube	100 each	RT
Color Card (milk sample)	1 each	RT

### **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 10 to 150 ppm is simple and sensitive as the reaction uses a chromophore that can be detected by eye. 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.



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The Nix Sensor or a spectrophotometer capable of reading at 510nm can be used to obtain quantitative results. If higher than expected concentrations of Benzoic Acid are detected, confirmation using analytical methods can be performed.

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 visual comparison of the reaction with the color scale derived from the Color Card.

Measuring range / color- Number of scale graduation	Number of determinations
10 – 20 – 40 – 60 – 80 – 100 – 150 ppm Benzoic Acid	100

### **Instructions:**

**Note:** Perform the reaction by mixing the following components in the specific order described below into one 1.5mL tube for each sample, positive and negative control (use a new pipet for each step).

Step 1. Pipet 50 µl of room temperature sample into 1.5mL tube

Step 2. Pipet 7 µl of Substrate

Step 3. Pipet 3 µl of Reaction Facilitator

Step 4. Pipet 30 µl of Chromophore

Step 5. Mix the components in the tube by pipetting up and down 3-4 times

Step 6. Incubate at room temperature for 5-10 minutes

Step 7. Read off the corresponding results in ppm by comparing to color card.

**Note:** The color card is specifically designed to be used to determine the relative Benzoic Acid concentration in your sample. If a different sample type is used, a negative control and spiked positive samples will need to be used for relative color reference. It is also possible to quantitate the Benzoic Acid concentration by measuring relational color with the QC Nix Color Sensor or absorbance at 510nm relative to a set of standards.

### **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, it is possible to set up a set of standards at known concentrations of Benzoic Acid which can be used to extrapolate the concentration in the sample being analyzed, loading into a 96 well plate and reading the samples at 510nm.



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A 1,500 ppm Benzoic Acid Solution is included in the kit to be used to produce sample spiked controls as needed. To spike a final concentration of 150 ppm of Benzoic Acid into 90µl reaction add 9µl of 1,500 ppm Benzoic Acid Solution.

**Notes on the measurement:** The color of the reaction may continue to change after the specified reaction time has elapsed. The rate of the reaction is impacted by the room temperature thus, incubating the plate in a set temperature incubator at 25°C, it can help ensure consistency.

**For Research Use Only. Not for use in diagnostic procedures.**