
Intended Use

The Beacon Chloramphenicol Plate Kit is an immunoassay for the detection of Chloramphenicol in food samples. This product is intended for research use only.

Principles

Calibrators and the Sample Extract(s) are pipetted into the test wells. After an incubation, Chloramphenicol HRP Enzyme Conjugate is added to each test well. During an incubation, Chloramphenicol in the calibrator/sample and Chloramphenicol HRP Enzyme Conjugate compete for binding to the polyclonal Chloramphenicol antibody immobilized on the test wells surface. Following the incubation, the wells are washed to remove any unbound Chloramphenicol and Chloramphenicol HRP Enzyme Conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the Chloramphenicol concentration of the sample is derived.

Reagents and Materials Provided

- 1 Plate containing 12 test strips of 8 wells each that are vacuum sealed in an aluminized pouch with a desiccant.
- 6 Vials of Chloramphenicol Calibrators (0, 0.025, 0.1, 0.6, 1.2, and 2 ppb).
- 1 Bottle of Chloramphenicol HRP Enzyme Conjugate.
- 1 Bottle of Sample Buffer.
- 1 Bottle of Substrate.
- 1 Bottle of Stop Solution.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s) (recommended when running more than two strips at once).
- Laboratory quality distilled or deionized water.
- Reagents and materials for sample preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Wash bottle (optional).
- Timer.
- Microtiter plate or strip reader capable of reading at 450 nm.

Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after expiration date.
- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (62°C to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- Each reagent is optimized for use in the Beacon Chloramphenicol Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Chloramphenicol Plate Kits with different lot numbers.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

Specificity

The Beacon Chloramphenicol Plate Kit cannot differentiate between the various Chloramphenicol's but detects their presence to differing degrees. The following table shows the percent cross reactivity of related compounds versus Chloramphenicol. All concentrations are in parts per billion (ppb).

Compound	% Cross-Reactivity
Chloramphenicol	100
Chloramphenicol Base	< 1
Thiamphenicol	< 1

Sample Preparation

Milk: (Dilution Factor: 1)

1. Measure 10 mL of sample into a glass centrifuge vial.
2. Centrifuge for 10 minutes at 3,000 x g at 4 – 12°C. If a refrigerated centrifuge is not available, chill the sample to 8°C prior to centrifugation.
3. Transfer the middle layer to a clean glass vial for use in the assay.

Milk Powder: (Dilution Factor: 4)

1. Weigh 2.5 g of sample into a glass centrifuge vial.
2. Measure 10 mL of laboratory quality distilled or deionized water and add to the vial. Vortex until the sample is completely dissolved.
3. Centrifuge for 10 minutes at 3,000 x g at 4 – 12°C.
4. Transfer the middle layer to a clean glass vial for use in the assay.

Honey: (Dilution Factor: 0.5)

1. Measure 3 g of sample into a glass centrifuge vial.
2. Measure 3 mL of laboratory quality distilled or deionized water and add to the vial. Mix to dissolve.
3. Measure 6 mL of ethyl acetate and add to the vial. Mix vigorously for 10 minutes.
4. Centrifuge for 10 minutes at 3,000 x g.
5. Transfer 2 mL of the supernatant to a clean conical tube.
6. Gently dry the extract using a weak nitrogen flow at 50°C.
7. Dissolve the dried sample in 0.5 mL Sample Buffer and use in the assay.

Egg: (Dilution Factor: 1)

1. Homogenized 100 g of sample.
2. Weigh 1 g of homogenized sample into a clean conical tube.
3. Measure 6 mL of ethyl acetate and add to the tube. Mix vigorously for 10 minutes.
4. Centrifuge for 10 minutes at 3,000 x g.
5. Transfer 3 mL of the supernatant to a clean conical tube.
6. Gently dry the extract using a weak nitrogen flow at 60°C.
7. Dissolve the dried sample in 1 mL isooctane/chloroform (2:3) mixture.
8. Add 0.5 mL of Sample Buffer. Vortex for 1 minute.
9. Centrifuge for 10 minutes at 3,000 x g.
10. Transfer the supernatant to a clean vial and use in the assay.

Meat Meal: (Dilution Factor: 0.5)

1. Homogenized 100 g of sample.
2. Weigh 3 g of homogenized sample into a clean conical tube.
3. Measure 6 mL of ethyl acetate and add to the tube. Mix vigorously for 10 minutes.
4. Centrifuge for 10 minutes at 3,000 x g.
5. Transfer 2 mL of the supernatant to a clean conical tube.
6. Gently dry the extract using a weak nitrogen flow at 60°C.
7. Dissolve the dried sample in 2 mL n-hexane/chloroform (2:3) mixture.
8. Add 0.5 mL of Sample Buffer. Vortex for 1 minute.
9. Centrifuge for 5 minutes at 3,000 x g.
10. Transfer the supernatant to a clean vial and use in the assay. If there is too much foam or gel in the intermediate phase and not enough fluid in the supernatant, put the sample in a heated water bath for 5 minutes at 80°C and centrifuge again.

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of test wells into a holder. Be sure to re-seal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **100 µL of Calibrators and Sample Extract(s)** into the appropriate well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
4. Incubate the test wells for **15 minutes** at room temperature.
5. Dispense **100 µL of Enzyme Conjugate** into each well.
6. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **15 minutes** at room temperature.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory quality distilled or deionized water and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
8. Dispense **100 µL of Substrate** into each well.
9. Incubate for **30 minutes** at room temperature.
10. Dispense **100 µL of Stop Solution** into each well in the same order of addition as the Substrate.
11. Gently shake the wells for 30 seconds using a back-and-forth motion.
12. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.

Result Interpretation

Semi-Quantitative Interpretation: Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrators:

- Samples with a lower absorbance (less color) than a calibrator have a concentration of Chloramphenicol greater than the concentration of the calibrator.
- Samples with a higher absorbance (more color) than a calibrator have a concentration less than the concentration of the calibrator.

Quantitative Interpretation: It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request. Please contact Beacon for further details.

- The concentration of Chloramphenicol in a sample is determined by comparing the average sample absorbance to the standard curve. This value must then be multiplied by the dilution factor used.
- Samples with absorbances lower than the highest calibrator contain a concentration of Chloramphenicol too high for quantification. Further dilute the sample extract in Sample Buffer to fit into the standard curve and retest along with the calibrators. Results must then be multiplied by the dilution factor used.
- Samples with Chloramphenicol absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.025 ppb or > 2 ppb, respectively.

Technical Assistance

For questions regarding this kit or for additional information about Beacon products, contact us.

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Safety

Stop Solution is 1N hydrochloric acid. Handle with care. To receive complete safety information on this product, contact Beacon Analytical Systems, Inc., and request Safety Data Sheets.

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