Chloramphenicol ELISA Kit

Catalog Number KA1415

96 assays

Version: 6.1

Intended for research use only

www.abnova.com

Table of Contents

Introduction

Intended Use

Enzyme Immunoassay for the determination of Chloramphenicol in sample extract.

Background

Chloramphenical (CPC) is an effective antibiotic against a wide range of bacteria and is especially useful in the treatment of gram-negative infections in cattle. Bone marrow depression, a potential precursor to aplastic anemia and other serious blood disorders, has been associated with chloramphenicol treatment of people already sensitized to the drug. The Food and Drug Administration have established a zero tolerance level for chloramphenicol residues in both domestic and imported animal products. The broad-spectrum activity of CPC and its low cost attracts its use by some food producers. Chloramphenicol may also be used in both the larval and grow out stages of cultured shrimp farm. Therefore, chloramphenicol could be present in shrimp tissue if its withdrawal periods prior to harvest are not appropriate monitored. The sensitivity of the Chloramphenicol ELISA kit is excellent, and is well suited for the determination of Chloramphenicol in sample extracts.

Principle of the Assay

The enzyme immunoassay for chloramphenicol is based on the competition between the chloramphenicol to be assayed and the chloramphenicol-alkaline phosphatase conjugate, for binding to rabbit antibody directed against chloramphenicol, coated onto microwells. The sample containing the chloramphenicol, and the chloramphenicol-alkaline phosphatase conjugate, when added to the microtiter wells, compete for binding to a limiting number of antibody sites. After incubation, each well is rinsed in order to remove non-bound components. The bound enzymatic activity is then measured by the addition of a chromogenic substrate. The intensity of the color developed is inversely proportional to the concentration of chloramphenicol in the sample. The concentration is calculated on the basis of a standard curve.

General Information

Materials Supplied

List of component

Component	Amount
96-wells microtiter plate (#S). Eight strips of 12 detachable wells, coated with rabbit Anti-Chloramphenicol antibody.	96 wells
Positive Calibrator: Containing 0 ng/mL, 0.25 ng/mL, 2.0 ng/mL and 10 ng/mL of chloramphenicol.	0.9 mL x 4
Chloramphenicol-alkaline phosphatase conjugate (CPC-ALP) (#3)	10.5 mL
p-Nitrophenyl phosphate (pNPP) substrate (#5): Ready to use.	10.5 mL
Wash Buffer (10xPBS-Tween) (#6): Dilute 10 fold with distilled or deionized water to 150 mL prior to use.	15 mL
Stop Solution (#7): 3 N NaOH.	5.5 mL

Storage Instruction

All reagents of the kit are stable, if stored at 2-8 ^{o}C, until the expiration date stated on the kit

Materials Required but Not Supplied

- ✓ Pipetters capable of delivering 50 µL and 100 µL.
- ✓ Microtiter plate reader (wavelength 405 nm).
- ✓ Plate washer or squeezable wash bottle.
- ✓ Timer.
- ✓ Absorbent paper towels.

Precautions for Use

Reagent are for in vitro research use only.

- ✓ Do not mix reagents from different lots.
- ✓ If concentrations of chloramphenicol in the samples are high, dilute sample such that points fall in the middle range of the standard curve.
- ✓ Do not return unused reagents back into their original bottles.
- ✓ Samples tested should have a pH of 7.0 (± 1.0). Excessive alkaline or acidic conditions may affect the test results.
- ✓ The stop solution contains NaOH. Do not allow to contact skin or eyes. If exposed, flush with water.
- ✓ Dispose of all materials, containers and devices in the appropriate receptacle after use.

Assay Protocol

Assay Procedure

Let the components of the kit equilibrate to room temperature before use.

1. Carefully add 50 µL of standard or sample to the bottom of each well. Slightly tap the side of the strip holder to evenly distribute the sample.
2. Avoid touching the well with pipette tip and add 100 µL of CPC-ALP conjugate (#3) to each well. Slightly tap the side of the strip holder to properly mix the sample and enzyme conjugate.
3. Incubate at room temperature for 40 minutes.
4. After incubation, dispose the solution in the wells by inverting and shaking. Wash microtiter wells 3 times with wash buffer to remove the non-bound conjugate. Washing may be done manually as follows: use squeeze bottle to fill wells gently with wash buffer, dumping the wells between each wash by inverting and shaking. After the third wash, tamp holder with washed strips onto a piece of absorbent paper.
5. Add 100 µL of pNPP substrate (#5) to each well and incubate at room temperature for 20 min. To avoid contamination, place the needed amount of substrate into a test tube and dispense only from the tube itself.
6. Add 50 µL of Stop Solution (#7) to each well and tap the strip holder for proper mixing.
7. Read absorbance at 405 nm using an ELISA reader.

- Simplified Assay Procedure

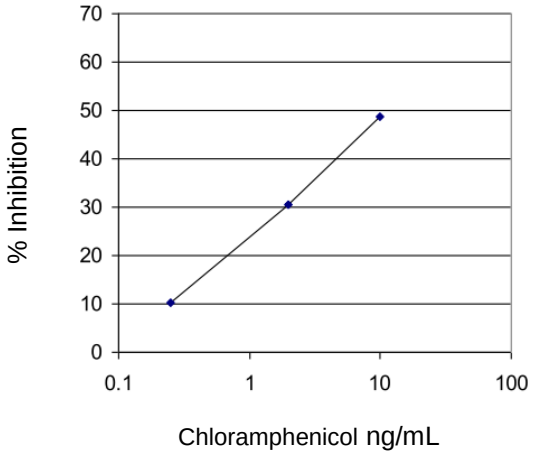
1. Add sample or standard (50 µL).
2. Add Enzyme conjugate (100 µL). 40 min at RT.
3. Wash 3x.
4. Add pNPP (100 µL), wait for 20 min. at RT.
5. Add stop solution (50 µL) and read at 405 nm.

Data Analysis

Calculation of Results

1. Calculation
   (a) Average the absorbance (OD_s) for each standard concentration of chloramphenicol including 0 ng/mL (OD_0).
   (b) % of Inhibition = 100 -(OD_s / OD_0) x100
2. Plot values of % of Inhibition, step 1 (b), against their corresponding concentrations on Log_{10} paper.
3. Calculate chloramphenicol concentration in the sample by interpolation and multiply by sample's dilution factor to obtain the actual quantity of chloramphenicol.

✓ Chloramphenicol Inhibition curve



Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H