ABSbio™ Chloramphenicol ELISA Kit (SE-CAP)

Chloramphenicol Quantification

Introduction

Chloramphenicol (CAP) is a broad spectrum antibiotic which was widely used in veterinary medicine. ABSbio™ Chloramphenicol ELISA Kit provide a rapid, sensitive and reliable assay for the determination of chloramphenicol in food. The High Sensitivity plate is coated with the antibody and pre-blocked to provide timesaving for high-throughput users. Chloramphenicol containing samples or standards and an antibody directed against chloramphenicol are given into the wells of the microtiter plate. The chloramphenicol contained in samples or standards will bind to the antibody which reacts with the binding protein coated onto the microtiter plate. After 60 minutes incubation at room temperature a chloramphenicol-peroxidase conjugate is added into the wells without a preceding washing step to saturate free antibody binding sites. After additional 30 minutes incubation at room temperature the wells are washed with diluted washing solution to remove unbound material. Finally, TMB substrates are added, incubated for detection, and a blue color is developed. Reaction is stopped and color turns to yellow when Stopping Solution (acidic) is added. The yellow color intensity proportionally correlates to the concentration of the chloramphenicol in samples.

Kit Components (96 tests)

Chloramphenicol Standard:	7 vials	Assay Diluent:	30 mL	TMB Solution:	16 mL	Plate Sealer:	2	Detection Antibody:	1 vial
HRP-Chloramphenicol:	1 vial	Wash Solution:	30 mL	Stop Solution:	12 mL			Antibody coated plate:	1

Storage and Handling: Shipping on ice. Store standard and antibody at -20℃, other components at 2-8 ℃. Shelf Life: 6 months after receipt. Warm up plate to room temperature before use.

Reagent Preparation

- After taking out from 2-8℃ refrigerator, prewarm the kit 30 minutes at room temperature before using. Secure the desired numbers of coated wells in the holder.
- **Wash Solution:** 10x dilute Wash Solution with dH_2O to prepare 1x Wash Solution.
- **Detection Antibody:** Immediately before use, add 60 µl of the anti-CAP ab into 6 mL of Assay Diluent for one plate (for partial plate assay, adjust the volumes accordingly).
- **HRP-chloramphenicol conjugate:** Immediately before use, add 60 µl of the conjugate into 6 mL of Assay Diluent for one plate (for partial plate assay, adjust the volumes accordingly).

Procedure Guideline

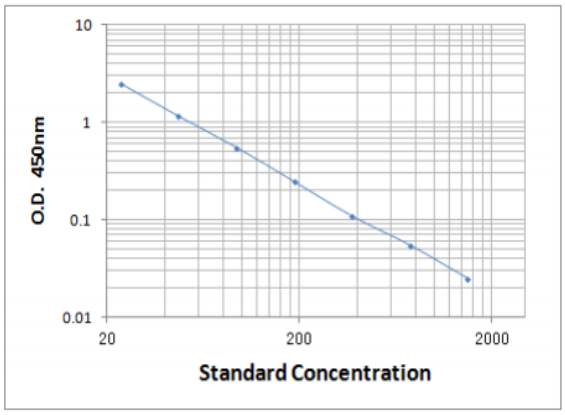
1. Prepare sample dilutions (3 or 10 fold) in a clean 96-well plate with Assay Diluent or 1x PBS.
2. Set standard wells, testing sample and blank wells on the assay plate/strip. Transfer diluted standard 50 µl to standard wells, 50 µl sample to sample wells, 50 µl sample diluent only to blank wells. Assay should be run in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	5ng/mL standard	5ng/mL standard	Undiluted sample 1	Undiluted sample 1								
B	2.5ng/mL	2.5ng/mL	3-fold	3-fold								
C	1ng/mL	1ng/mL	Undiluted sample 2	Undiluted sample 2								
D	0.5ng/mL	0.5ng/mL	3-fold	3-fold								
E	0.1ng/mL	0.1ng/mL	Undiluted sample 3	Undiluted sample 3								
F	0.05ng/mL	0.05ng/mL	3-fold	3-fold								
G	0.025ng/mL	0.025ng/mL	Undiluted sample 4	Undiluted sample 4								
H	blank	blank	3-fold	3-fold								

3. Immediately add 50 µl diluted anti-CAP ab into each well, and cover the plate with plate sealer, incubate the plate at room temperature for 1 hr on an oscillating shaker.
4. Add 50 µl of the prepared HRP-Chloramphenicol conjugate into each wells without preceding washing.
5. Cover the plate with plate sealer and incubate at room temperature for 0.5 hour.
6. Decant as much liquid as possible, fill the wells with 250 µl wash solution, oscillate the plate on an oscillating shaker if available for 1 min, decant the wash solution and remove residual liquid with absorbent paper. Repeat wash five times.
7. Add 150 µl of TMB Solution to each well and incubate at room temperature for 10~30 minutes, or keep close monitoring on the developing process until desired developing blue color observed.
8. Add 50 µl of Stop Solution to each well to stop the reaction (the blue color change to yellow).
9. Read absorbance of the plate on a microplate reader at 450 nm within 15 min.
10. Average the duplicate OD readings for each standard and sample. Create a standard curve by subtracting the mean OD's for the blank. Construct standard curve (plotting the mean OD450 for each standard on the Y-axis against concentration on the X-axis, draw a best-fit curve through the points) and calculate linear regression equation, then use sample OD values and regression equation to calculate the corresponding sample concentration. It should be remembered that the sample has been diluted and its actual concentration should be justified by dilution factor (the measurement and calculation can be accomplished by software like SoftMax).

Typical Standard Curve

Always run your own standard curves for calculation of results.



Quantification of Chloramphenicol (CAP).

Standard, Samples and Anti-Cap ab were incubated at room temperature for 1 hours in the coated plate. For ELISA analysis using Chloramphenicol (CAP) ELISA Kit (#SE-CAP), the Chloramphenicol-HRP conjugate was incubated for another 0.5 hour at room temperature. Following five wash steps. The peroxidase was detected by using a soluble TMB solution for ELISA applications. The reaction was stopped using stop solution. Absorbance was read on a Molecular Devices SpectraMax® at 450 nm.

Sample collection and storage

Milk (direct assay)

- Refrigerate fresh milk samples at 2-8℃ and followed by centrifugation at 3000 g for 10 min
- Remove the upper fat layer and test the sample directly for the ELISA after warming to room temperature

Honey

- Dissolve 2 g honey in 4 ml double distilled water. Add 4 ml ethyl acetate and agitate vigorously for 10 minutes.
- Transfer 1 ml of the upper ethyl acetate phase to a clean glass vial and evaporate the solvent at 50-70℃ under a nitrogen stream to dryness.
- Dissolve the dry residue with 500 µl sample diluent by shaking vigorously and test the sample for the ELISA.

Shrimps, Meat, Fish

- Homogenize sample with an appropriate device. Mix 3 g sample with 3 mL double distilled water, add 6 ml ethyl acetate and agitate vigorously for 10 minutes. Centrifuge for 10 minutes at 3000 g (room temperature).
- Transfer 4 ml of the upper ethyl acetate phase to a clean glass vial and evaporate the solvent at 50-70℃ under a nitrogen stream to dryness.
- Add 1 ml n-hexane to the residue. Add 500 µl sample diluent to the mixture and agitate vigorously for 1 minute. Centrifuge for 10 minutes at 3000 g (room temperature).
- Take the lower, aqueous phase for the ELISA.

Performance Data

Standard range 0- 5 ng/mL	**Sensitivity 0.03 ng/mL**
Assay time 2 hours	**Validity Six months**
Coefficients of variation (CVs) for Std <10%;	**CVs for samples: <15%**

Cross-reactivities: Chloramphenicol 100%; Chloramphenicol glucuronide 25%; Thiamphenicol <1%

Related Products:

Furazolidone ELISA Kit (#SE-AOZ)	**Tetracycline ELISA Kit (#SE-TET)**
Chlortetracycline ELISA Kit (#SE-CTC)	**Sulfonamides ELISA Kit (#SE-SAS)**

TROUBLE SHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure that all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with washing solution.