Chloramphenicol Fast ELISA (Catalog: TBS21121)

One-step-One-Hour ELISA For determination of chloramphenicol concentrations in food and seafood samples

Description

Chloramphenicol is a broad-spectrum antibiotic effective against a wide variety of Gram-positive and Gram-negative bacteria. It is no longer commonly prescribed to patients because of the presence of resistant bacteria and safety concerns. One of its most prominent adverse effects is bone marrow toxicity, which includes direct bone marrow suppression and aplastic anemia, with the latter being relatively rare but unpredictable, not dosage-dependent, and generally fatal. Chloramphenicol is especially toxic for neonates. However, due to its low cost and effectiveness to improve yield, Chloramphenicol has been illegally used in agriculture and therefore is present in some seafood such as shrimp or crawfish. There are reports on its presence in milk, honey, and meat products as well.

Tribioscience's Chloramphenicol Fast ELISA Kit provides a flexible accurate, sensitive, and time-saving approach to determining the presence of Chloramphenicol in animal tissue (e.g. chicken, beef, fish, or shrimp), honey, or milk, providing a vital tool to prevent consumption of food tainted with Chloramphenicol.

Intended Use

The Chloramphenicol ELISA Kit utilizes competitive ELISA for the quantitative and qualitative analysis of Chloramphenicol levels in food samples including animal tissue (e.g. chicken, beef, fish, or shrimp), honey and milk. The limit of detection (LOD) of Chloramphenicol is 0.05 ppb (0.05 ng/mL).

Safety Instructions

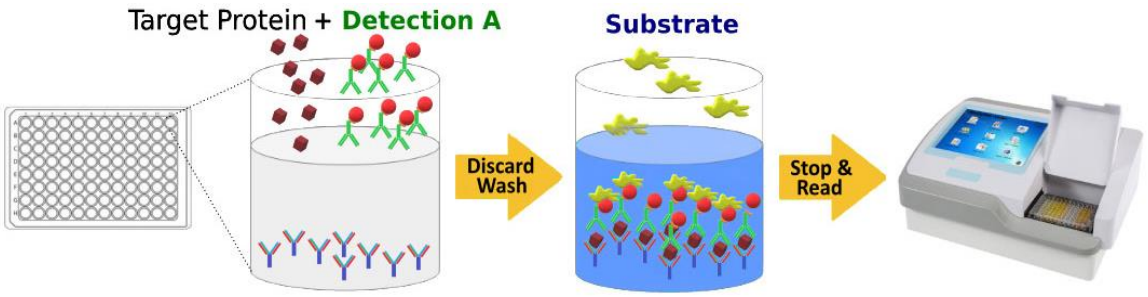
To receive complete safety information on this product, contact Tribioscience, Inc. and request Material Safety Data Sheets (MSDS).

Assay Principle

Tribioscience's Chloramphenicol ELISA Kit is a competitive enzyme-labeled immunoassay (Fig. 1). The 96- well microtiter plate is pre-coated with an anti-chloramphenicol antibody. During the assay, chloramphenicol standard solution or samples are added to each well, followed by adding horse radish peroxidase (HRP) -chloramphenicol conjugate, which will compete with chloramphenicol in standard or sample for binding to antibody during the incubation. After plate wash, an ultra-sensitive HRP substrate is added to the wells leading to a colored product only in the presence of HRP, and optical density is inversely related to chloramphenicol concentrations in the samples. The accurate concentration of chloramphenicol can then be determined by using the standard curve constructed in the same run.

The kit main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 1 hour, no need for 4-5 hours.

Fig. 1. Simple Procedures



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Pre coated Microplate	TBS21121A	96 well microplate (12 strips of 8 wells) coated with an antibody specific for chloramphenicol.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-
Chloramphenicol Standard	TBS21121B	40 µL of chloramphenicol (81 ng/mL, 20x).	Aliquot and store at -20 ℃ for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS21121C	80 µL of HRP- chloramphenicol conjugate (100x)	May be stored for up to 4 months at 2-8 °C.
Assay Diluent	TBS21121D	25 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 mL of concentrated solution (10x).	
TMB Substrate	TBS3000T	10 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 1 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

Sample preparation
Meat (including fish and shrimp) samples need to be processed as followings before assay:
1. Homogenize sample
2. Weight 1.0 g homogenized sample
3. Add 2ml Ethyl Acetate, mix by vortex thoroughly
4. Centrifuge 4000rpm for 10 min at room temperature
5. Take 1 ml supernatant
6. Evaporate to dryness in a nitrogen evaporator
7. Add 0.5 ml hexane to reconstitute
8. Add 0.5 ml of sample diluent
9. Vortex vigorously
10. Centrifuge 4000rpm for 10 min at room temperature
11. Discard upper layer, take 50 µl bottom aqueous phase to ELISA plate.

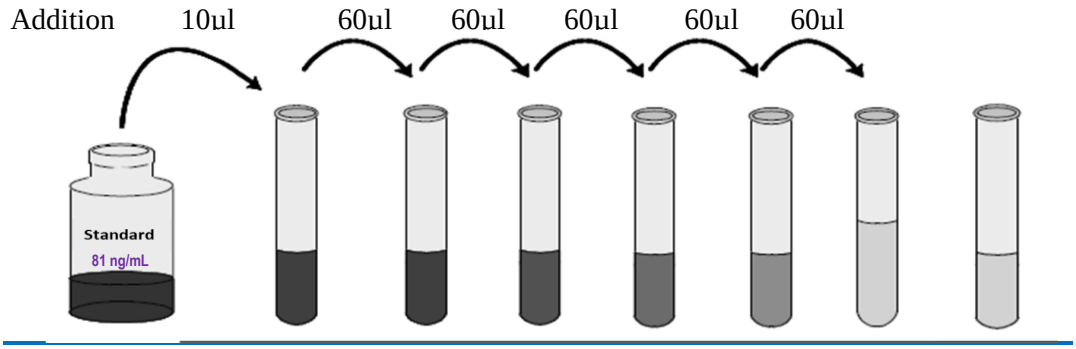
Assay Procedure
Equilibrate kit components at room temperature (20-25 °C) for at least 30 min prior to running the test, and thoroughly mix all liquid components before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate(10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer.

HRP-Chloramphenicol Conjugate: Prepare HRP working solution by diluting 1 part of HRP conjugate (100x) with 99 parts of Assay Diluent (1x) to HRP working solution (1x). For 1 plate, add 60 µL HRP-conjugate (100x) to 6 mL Assay Diluent.

Chloramphenicol Standard Preparation:
1. Label test tubes as #1 through #7.
2. Pipet 190 µL of 1x Assay Diluent into tube #1, and 120 µL into tubes #2 to #7 as diagram below (Fig.2).
3. Add 10 µL of the total aflatoxin Standard stock solution (81ng/mL) to tube #1(4.05ng/mL) and mix.
4. Make 3x serial dilutions of the standard using the Tube#1(4.05ng/mL standard solution) from Tube #2 through #6 with sequential transfer of 60 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 6 will be 4.05, 1.35, 0.45, 0.15, 0.05, 0.017ng/mL. Tube# 7 is Standard 0.

Fig.2 Diagram for Human standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7
Assay Buffer (µL)	190	120	120	120	120	120	10
Addition	Stock	Std1	Std2	Std3	Std4	Std5	
Addition Vol. (µL)	10	60	60	60	60	60	0
Final Conc (ng/ml)	4.05	1.35	0.45	0.15	0.05	0.017	0

Assay Procedures:

1. Add 50 µL of standard, sample, or control per well.
2. Add 50 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 30min**.
3. Aspirate each well, and wash for 3 times by filling each well with 200 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue.
5. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
6. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Quantitative Calculation of Chloramphenicol Concentration

A: Calculate B/B0: Dividing average absorbance of each standard and sample (B) by absorbance of the control of 0 ng/mL chloramphenicol concentration, B0) to obtain percentage absorbance.

Percentage absorbance (%) = 100%*(B/B_0).

B: Average absorbance of a standard or sample.
B_0: Average absorbance of 0 ng/mL control.

B: A standard curve is obtained by graphing the percentage absorbance of standards (Y axis) versus their corresponding concentration (X axis), and chloramphenicol concentration of can be read from this standard curve. Alternatively, chloramphenicol concentration in the samples can be calculated with regression equation correlating percentage absorbance to chloramphenicol concentration. Graphing software can also be used for quick analyses of samples.

Typical Data

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

Range of Standard Curve:	0 – 4.05 ng/ml
Assay Quantitative Range:	0.05 – 4.05 ng/ml
Assay Time:	50 min

Limit of Detection (LOD):
Meat (after calculation of dilution factor): 0.1 ppb.

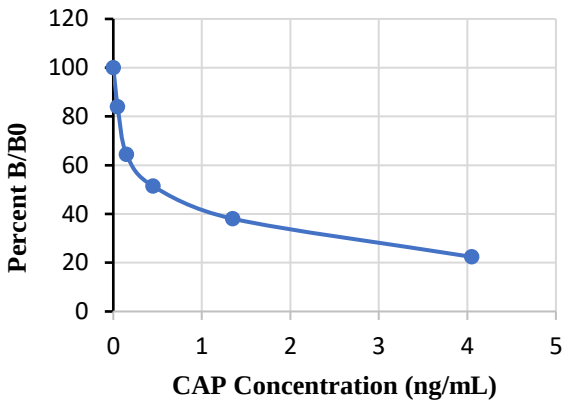
Recovery: 80-130%

Sensitivity (defined as the average of absorbance from 6 zero-standards minus 3 times of standard deviation): 0.015 ng/ml.

Precision:

Intra-assay CV	<10%
Inter-assay CV	<15%

Fig.3: CAP Standard Curve



Precautions

1. Assay kit should be stored at 2-8℃ and avoid freezing conditions; unused test strips should be sealed in re-closable bag; colorless substrate is sensitive to light so prolonged exposure to light needs to be avoided.
2. Reagents should be brought to room temperature (20-25℃) prior to use. A room temperature of lower than 20℃ or failure to equilibrate reagents or samples to room temperature could result in low OD readings for all samples. All reagents should be put back into 2-8℃ storage immediately after use.
3. Do not use reagents beyond expiration date.

Storage and Expiration Date

Storage: All components of the kit should be stored at 2-8℃. Expiration Date: This kit expires 12 months after manufacturing date.

Technical Assistance

For ordering or technical assistance regarding this kit, or for additional information about Tribioscience products, please email: support@tribioscience.com or call (408) 498-0197, or 833-697-8998 (Toll Free).

For research use only.