Product Manual

Chloramphenicol Competitive ELISA Kit

Catalog Number

MET-5139 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Chloramphenicol is an antibiotic that has been used to treat various types of bacterial infections such as conjunctivitis, meningitis, plague, cholera, and typhoid fever. Chloramphenicol is considered to be a broad-spectrum antibiotic that halts bacterial growth by inhibiting protein production.

Usual side effects of chloramphenicol treatment may include nausea, myelosuppression (suppression of leukocyte, erythrocyte, or thrombocyte production), and diarrhea. Myelosuppression can lead to death and therefore treatment time is usually reduced to an absolute minimum. People with pre-existing liver or kidney problems may need lower treatment doses. A condition in young children known as gray baby syndrome may occur (the syndrome can cause swollen stomach and low blood pressure). Chloramphenicol use is usually not recommended near the end of pregnancy or while breastfeeding.

Cell Biolabs' Chloramphenicol Competitive ELISA Kit provides a convenient method for the detection of total chloramphenicol in extracts from cells, tissue, serum, plasma, or foods. The total content of chloramphenicol in unknown samples is determined by comparison with a chloramphenicol standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples. The kit has a detection sensitivity limit of 370 pM chloramphenicol.

Assay Principle

First, a chloramphenicol conjugate is coated on an ELISA plate. The unknown chloramphenicol samples or chloramphenicol standards are then added to the chloramphenicol conjugate preabsorbed ELISA plate. After a brief incubation, an anti-chloramphenicol antibody is added, followed by an HRP conjugated secondary antibody. The total content of chloramphenicol in unknown extracted samples is determined by comparison with a chloramphenicol standard curve.

Related Products

1. MET-5135: Gentamicin Competitive ELISA Kit
2. MET-5144: Kanamycin Competitive ELISA Kit
3. AKR-110: Rapid GST Inclusion Body Solubilization and Renaturation Kit

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-Chloramphenicol Antibody (500X) (Part No. 51391C): One 10 µL vial of anti-Chloramphenicol Antibody.
3. Secondary Antibody-HRP Conjugate (Part No. 51394C): One 10 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.

7. Stop Solution (Part No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Chloramphenicol Standard (Part No. 51392C): One 50 µL vial of 150 µM Chloramphenicol.
2. Chloramphenicol Conjugate (500X) (Part No. 51393C): One 25 µL vial.
3. 100X Conjugate Diluent (Part No. 281603): One 300 µL vial.

Materials Not Supplied

1. 1X PBS
2. Bovine Serum Albumin (BSA)
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wavelength)

Storage

Upon receipt, store Anti-Chloramphenicol Antibody (500X), Secondary Antibody-HRP Conjugate, Chloramphenicol Standard, 100X Conjugate Diluent, and Chloramphenicol Conjugate at -20ºC. Store all the remaining components at 4°C.

Preparation of Reagents

- Chloramphenicol Conjugate Coated Plate:

  Note: The Chloramphenicol Conjugate coated wells are not stable and should be used within 24 hours after coating. Only coat the number of wells to be used immediately.

  1. Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 50 µL to 4.95 mL of 1X PBS.
  2. Immediately before use, prepare 1X Chloramphenicol Conjugate by diluting the 500X Chloramphenicol Conjugate in 1X Conjugate Diluent. Example: Add 10 µL of 500X Chloramphenicol Conjugate to 4.99 mL of 1X Conjugate Diluent.
  3. Add 100 µL of the 1X Chloramphenicol Conjugate to each well to be tested and incubate overnight at 4ºC. Remove the Chloramphenicol Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature on an orbital shaker. Transfer the plate to 4ºC and remove the Assay Diluent **immediately before use.**
- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-Chloramphenicol Antibody and Secondary Antibody: Immediately before use, dilute the Anti-Chloramphenicol antibody 1:500 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of Chloramphenicol standards in the concentration range of 0 to 1500 nM by diluting the Chloramphenicol Standard in Assay Diluent (Table 1).

Standard Tubes	150 µM Chloramphenicol Standard (µL)	Assay Diluent (µL)	Chloramphenicol (nM)
1	5	495	1500
2	100 of Tube #1	300	375
3	100 of Tube #2	300	94
4	100 of Tube #3	300	23
5	100 of Tube #4	300	6
6	100 of Tube #5	300	1.5
7	100 of Tube #6	300	0.37
8	0	300	0

Table 1. Preparation of Chloramphenicol Standards

Preparation of Samples

- Serum: Avoid hemolyzed and lipemic blood samples. Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in Assay Diluent as necessary.
- Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with heparin or citrate and centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in Assay Diluent as necessary.
- Cells or tissues: Homogenize 50-200 mg of the cell pellet or tissue in 0.5-2 mL of ice cold PBS using a mortar and pestle or by dounce homogenization. Incubate the homogenate at 4°C for 20 minutes. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Recover the supernatant and transfer to a fresh tube. Store resuspended sample at -20°C or colder. Perform dilutions in Assay Diluent as necessary.
- Food samples: Homogenize 1-5 grams using a mortar and pestle or by dounce homogenization. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Store homogenized sample at -20°C or colder. Perform dilutions in Assay Diluent as necessary.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each Chloramphenicol sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or chloramphenicol standard to the wells of the Chloramphenicol Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted anti-Chloramphenicol antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash 3 times with 250 µL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 3 times according to step 4 above.
6. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 2-20 minutes on an orbital shaker.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
7. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
8. Read absorbance of each well on a microplate reader using 450 nm as the primary wavelength.

Example of Results

The following figures demonstrate typical Chloramphenicol Competitive ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

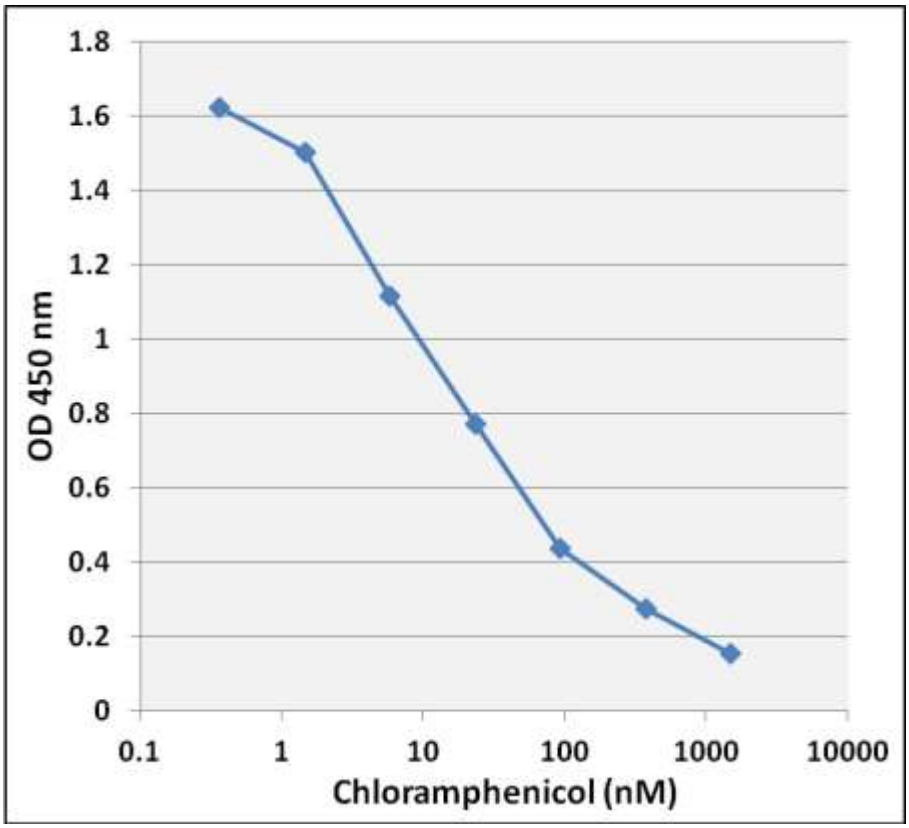

Figure 1: Chloramphenicol Standard Curve.

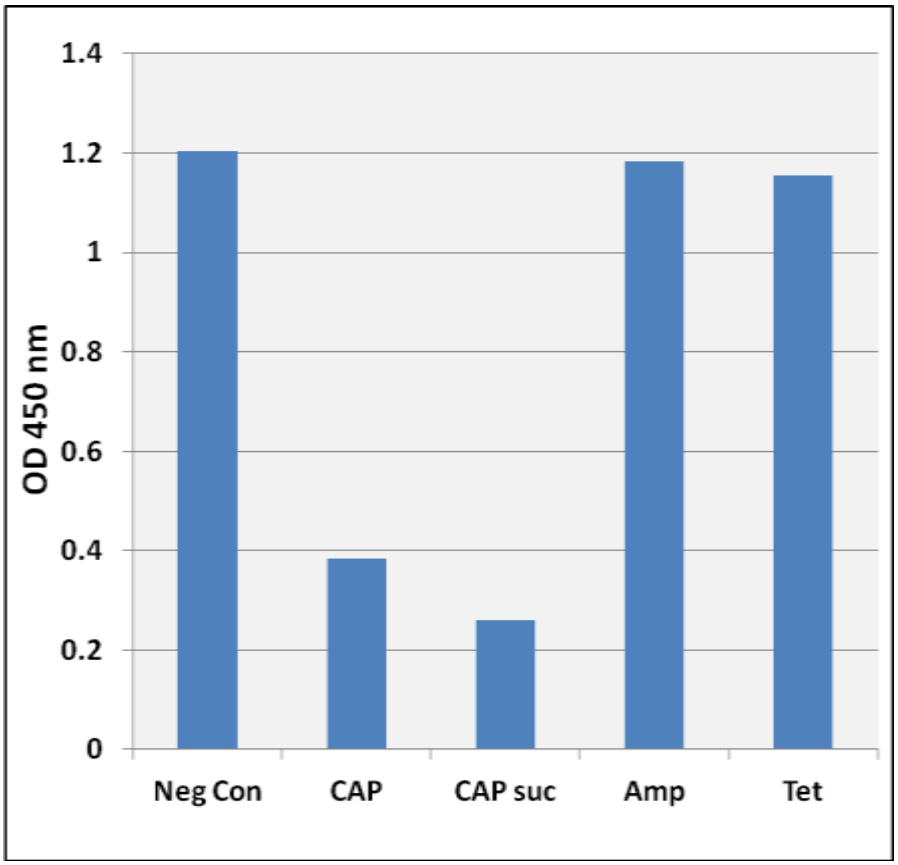

Figure 2: Specificity of Chloramphenicol ELISA. No drug (Neg Con), 375 nM chloramphenicol (CAP), chloramphenicol succinate (CAP suc), Ampicillin (Amp) or Tetracycline (Tet) was measured using the Chloramphenicol Competitive ELISA Kit.

References

1. Feder HM Jr, Osier C, and Maderazo EG (1981) *Rev Infect Dis*. **3**:479-491.
2. Balbi HJ (2004) *Pediatr. Rev*. **25**:284-8.
3. Feder, HM Jr. (1986). *South Med. J.* **79**:1129–1134.
4. Mulhall, A, de Louvois J, and Hurley R. (1983). *British Med J. (Clin Res Ed)*. **287**: 1424–1427.
5. Park, JY, Kim KA, and Kim SL (2003). *Antimicrob. Agent. Chemother*. **47**: 3464–3469

Warranty

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com