Antibiotics ELISA kits available from ADI:

http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2739

DE-100010	Clenbuterol ELISA kit, 96 tests (For Urine, Serum, Feed, Meat, Liver)
DE-100020	Ractopamine ELISA kit, (For Liver, Urine, Feed), 96 tests
DE-100030	Salbutamal ELISA kit, For Urine, Tissue, Feed, Animal Tissue, Aquatic, Honey, Intestine,, 96 tests
DE-100040	Chloramphenicol ELISA kit, 96 tests (For Animal Tissue, Aquatic, Honey, Intestine, Urine, Egg, Milk, Serum)
DE-100050	Florfenicol ELISA kit (For Animal Tissue, Aquatic, Honey), 96 tests
DE-100060	Nitrofuran (AMOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100070	Nitrofuran (AHD) ELISA kit, (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100075	Nitrofuran (SEM) ELISA kit (Honey, Fish, Shrimp, Chicken/Liver, Fish/Shrimp), 96 tests
DE-100080	Nitrofuran (AOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100090	Sulfonamides Residues (SAs) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests
DE-100100	Sulfamethazine (SM2) ELISA kit, 96 tests (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk)
DE-100110	Sulfamethoxydiazine (SMD) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine), 96 tests
DE-100120	Quinolones (QNS) ELISA kit (For Pork/Liver, Chicken/Liver, Shrimp, Fish, Serum, Honey), 96 tests
DE-100130	Enrofloxacin ELISA kit (For Pork/Liver, Chicken/Liver, Shrimp, Fish, Serum, Honey), 96 tests
DE-100140	Ampicillin ELISA kit, (For Pork/Liver, Chicken, Duck, Shrimp, Fish, Honey, Milk), 96 tests
DE-100150	Benzyl Penicillin ELISA kit, (For Pork/Liver, Chicken, Duck, Shrimp, Fish, Honey, Milk), 96 tests
DE-100160	Tylosin ELISA kit (For Meat, Liver, Honey, Egg), 96 tests
DE-100170	Trenbolone ELISA kit (For Animal Tissue, Aquatic, Urine), 96 tests
DE-100180	Diazepam ELISA kit (For Tissue, Urine, Feed), 96 tests
DE-100190	Diethylstilbestrol (DES) ELISA kit (Fish, Shrimp, Liver, Meat, Feed, Urine), 96 tests
DE-100200	Gentamicin ELISA kit (Chicken/Liver), 96 tests
DE-100210	Streptomycin ELISA kit, 96 tests (Chicken/Liver, Honey, Milk)
DE-100230	Olaquindox ELISA kit (Tissue) 96 tests
DE-100240	Sulfaquin-oxaline ELISA kit, (For Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests

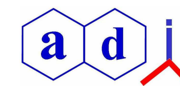
See Details at the web site or Contact ADI

Instruction Manual No. M- DE-100040

Chloramphenicol 0.05 ppb (CAP) ELISA KIT

Cat. #. DE-100040

For Qualitative Quantitative Determination
of CAP in animal tissue, aquatic, honey, intestine, urine, egg, milk and serum.



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Web: www.atzlabs.com

Chloramphenicol (CAP) ELISA KIT Cat. # DE-100040

Kit Components, 96 tests	Cat #
Micro-well coated strip plate (12 strips with 8 removable wells each)	DE-100041
6x standard solution (1ml each): 0.0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb and 4.05ppb	DE-100042
Enzyme conjugate (12ml)	DE-100043
Antibody concentrated solution (1ml)	DE-100044
Substrate A solution (7ml)	DE-SSA
Substrate B solution (7ml)	DE-SSB
Stop solution (7ml)	DE-ST
20x Concentrated Washing buffer (40ml)	DE-WB
2x Concentrated redissolving solution (50ml)	DE-SS2
Instruction Manual	M- DE-100040

INTRODUCTION

Chloramphenicol (CAP) is an antibiotic originally derived from the bacterium Streptomyces venezuelae isolated first by David Gottlieb. Streptomyces venezuelae was collected from a sample in Venezuela in 1947. Chloramphenicol chemical formula is $C_{11}H_{12}Cl_2N_2O_5$, its molecular weight is 323.132 g/mol was the first antibiotic to be synthetically produced on a large scale. It is widely used against a variety of microorganisms, especially in low income countries because of the low cost production. The oral use in the West is not very popular due to the little possibility to cause aplastic anemia on rare occasions. However, it is still topically used against eye infections. Researchers found that chloramphenicol can also work against the fungus Batrachochytrium dendrobatidis that cause a disease called chytridiomycosis. Chytridiomycosis is a fungal disease that has been responsible for the extinction of one-third of 120 species of frogs lost since 1980.

Chloramphenicol inhibits the synthesis of essential protein for the bacteria, and in some eukaryotic cells by binding to the 50S subunit of the ribosome. Without these proteins the bacteria cannot grow and replicate. The drug enters the bacterial cells by facilitated diffusion. Using this mechanism eye drops include chloramphenicol to treat a bacterial eye infection called conjunctivitis. Chloramphenicol works against Gram-positive bacteria, Gram-negative bacteria and anaerobes bacteria. It is active against Rickettsia, Chlamydia, and Mycoplasma. It is mainly effective against H. influenzae, S. pneumoniae, S. typhi, and Neisseria species. Chloramphenicol has a great solubility in cerebrospinal fluid so it is used to treat staphylococcal brain absecesses.

Alpha Diagnostic Intl's Chloramphenicol (CAP) ELISA kit is a highly sensitive competitive type assay for the measurement of CAP in animal tissue, aquatic, honey, intestine, urine, egg, milk and serum.

CALCULATION OF RESULTS

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the content of Chloramphenicol.

Qualitative determination

The concentration range (ng/mL) can be obtained from the comparison the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.250, and that of the sample Ⅱ is 0.720, while those of the standard solutions are as the followings: 1.610 for 0 ppb, 1.380 for 0.05 ppb, 1.100for 0.15 ppb, 0.620 for 0.45 ppb, 0.289 for 1.35 ppb and 0.108 for 4.05 ppb, accordingly the concentration range of the sample Ⅰ is 1.35 to 4.05 ppb, and that of the sample Ⅱ is 0.15 to 0.45 ppb.

Quantitative determination

The mean values of the absorbance values is obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

$$\text{Percentage of absorbance value} = \frac{B}{B0} \times 100\%$$

B—the average OD value of the sample or the standard solution
B0—the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the Chloramphenicol standard solution (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, thus finally obtaining the Chloramphenicol concentration in the sample.
Using the professional analyzing software of this kit will be more convenient for the accurate and
rapid analysis of a large amount of samples. (Please contact us for this software)

Technical specifications

Sensitivity: 0.05 ppb
Detection limit
- Egg, intestine........0.1 ppb
- Honey........0.15 ppb
- Milk........0.05 ppb
- Urine and serum........0.1 ppb
- Chicken, pork, fish, shrimp........0.05 ppb

Recovery rate
- Chicken, pork, fish, shrimp........ 80%±10%
- Honey........70%±10%
- Milk........85%±15%

Cross-reaction rate
- Chloramphenicol........ 100%
- Thiamphenicol........< 0.1%
- Florfeniol........< 0.1%

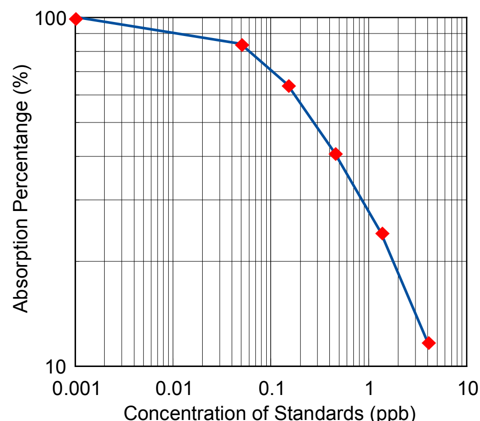
Precautions

1. The room temperature below 20 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility.
3. Mix every reagent and reaction mixture evenly and wash the microplate thoroughly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin;
5. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colorless color former is light sensitive, and thus they cannot be directly exposed to the light.
6. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.
7. Discard the coloration solution with any color that indicates the degeneration of this solution. The detecting value of the standard solution 1 (0 ppb) of less than 0.5 indicates its degeneration.
8. The optimum reaction temperature is 25 ℃, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

Work Sheet of Typical Assay-Chloramphenicol

Wells	Stds/samples	Mean $A_{450\ nm}$	Absorption Percentage
A1, A2	**Standard A** 0.0 ppb	1.610	100%
B1, B2	**Standard B** 0.05 ppb	1.380	85.71%
C1, C2	**Standard C** 0.15 ppb	1.100	68.32%
D1, D2	**Standard D** 0.45 ppb	0.620	38.51%
E1, E2	**Standard E** 1.35 ppb	0.289	17.95%
F1, F2	**Standard F** 4.05 ppb	0.108	6.71%

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



NOTE: A typical assay Standard Curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

This test kit is based on the competitive enzyme immunoassay for the detection of Chloramphenicol in the sample. The coupling antigen is pre-coated on the micro-well stripes. The Chloramphenicol in the sample and the coupling antigen pre-coated on the micro-well stripes compete for the anti-Chloramphenicol antibody. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Chloramphenicol in it. This value is compared to the standard curve and the Chloramphenicol concentration is subsequently obtained.

MATERIALS AND EQUIPMENT REQUIRED

Equipments: microplate reader(450nm), homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipettes

Micropipettors: single-channel 20 to 200 µL and 100 to 1000 µL, and multi-channel 250 µL.

Reagents: Ethyl acetate, N-hexane or N-heptane.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Chloramphenicol Kit is for research use only.

Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid, if not already on file, can be requested or obtained from the ADI website.

SAMPLE PRE-TREATMENT

Instructions

The following points must be dealt with before the pre-treatment of any kind of sample:

1. Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.
2. Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.
3. In the course of sample pre-treatment, operate strictly by manual, and wipe off all the organic solvent before distilled solution adding to micro-wells so as to lead to false positive.

Redissolving solution: dilute 10× concentrated redissolving solution at 1:10 (1+9) with deionized water.

Honey

1. Put 2.0 g honey into centrifuge vessel, add 4 mL of the deionized water to dilute.
2. Add 4 mL ethyl acetate, shake upside down for 10 min.
3. Centrifuge at 4000g at room temperature (20-25 ℃) for 10 min.
4. Transfer 1 mL supernate into a new vessel, evaporate to dryness by nitrogen at 60 ℃.
5. Dissolve the dry residue in 0.5 mL of the redissolving solution.
6. Take 80 µL for further analysis.

Milk and milk powder

1. Centrifuge sample(if milk powder, firstly diluted at 1:6 with the deionized water, that's to say, 1 g milk powder in 6 mL deionized water) at above 4000 g at 10 ℃ for 10 min. Remove from the fat of super layer.
2. Put 4 mL sample into centrifuge vessel. add 8 mL Ethyl acetate, and mix thoroughly, centrifuge at above 4000 g at room temperature for 10 min.
3. Take 6 mL supernate to a new centrifuge vessel. Blow to dry with nitrogen at 60 ℃ completely.
4. Dissolve the dry residue in 0.3 mL of the redissolving solution.
5. Take 80 μL for further analysis.

Shrimp, fish and meat sample

1. Remove and homogenize the sample.
2. Take 3 g sample into vessel, add 6 mL ethyl acetate, shake properly for 10 min, centrifuge at above 4000 g at room temperature (20-25℃) for 10 min.
3. Take 4 mL of the supernate (equivalent to 2 g sample) to a new vessel, and evaporate to dryness by nitrogen in 60℃.
4. Dissolve the dry residues in 1 mL redissolving solution. add 1 mL N-hexane, shake vigorously, centrifuge at above 4000 g at room temperature for 10 min.
5. After centrifugate, if it appears emulsification, take the vessel in 80℃water bath for 5 min, then centrifugate. Remove N-hexane of upper layer absolutely. Take 80 μL of the lower for analysis.

Serum and plasma

1. Transfer 1 mL serum or plasma into vessel, add 2 mL ethyl acetate, shake properly for 10 min.
2. Centrifuge at 4000 g at room temperature for 10 min.
3. Transfer 1 mL supernate (equivalent to 0.5 mL sample) into a new vessel and evaporate to dryness by nitrogen.
4. Dissolve the dry residue in 0.5 mL of the redissolving solution.
5. Take 100 μL for further analysis.

Egg

1. Homogenize the sample (including albumen and yolk).
2. Take 1 g sample into a centrifuge vessel. add 6 mL Ethyl acetate, and mix thoroughly for 10 min, centrifuge at above 4000 g at room temperature for 10 min.
3. Transfer 3 mL supernate (equivalent to 0.5 g sample) into a centrifuge vessel, blow to dry with nitrogen at 60 ℃.
4. Dissolve dry residues in 0.5 mL redissolving solution, and add 1 mL N-hexane, mix properly, centrifuge at above 4000 g at room temperature (25±2 ℃) for 10 min.
5. After centrifugate, if it appears emulsification, take the vessel in 80℃water bath for 5 min, then centrifugate. Remove N-hexane of upper layer absolutely. Take 80 μL of the lower for analysis.

Urine

1. Take 0.5 mL Urine, and add 2 mL redissolving solution, then mix properly.
2. Centrifuge at 4000g at room temperature for 10 min.
3. Take 80 μL for further analysis.

Feed

1. Take 1 g sample to a vessel, and add 4 mL Ethyl acetate, then mix properly. Centrifuge at 4000g at room temperature for 10 min.
2. Transfer 1 mL supernate(equivalent to 0.25 g sample) into a centrifuge vessel, blow to dry with nitrogen at 60 ℃.
3. Dissolve dry residues in 0.5 mL redissolving solution, and add 1 mL N-hexane, mix properly, centrifuge at above 4000 g at room temperature (25±2 ℃) for 10 min.
4. After centrifugate, if it appears emulsification, take the vessel in 80℃water bath for 5 min, then centrifugate. Remove N-hexane of upper layer absolutely. Take 80 μL of the lower for analysis.

STORAGE AND STABILITY

Storage: store at 2 to 8 ℃, not frozen.

Expiration date: 12 months; date of production is on the box.

Instructions

1. Bring all reagents and micro-well strips to the room temperature (20-25 ℃) before use (about 2 h);
2. Return all reagents to 2-8 ℃ immediately after use.
3. Don't change analysis procedures.
4. Don't interrupt any operation procedures.
5. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in ELISA the procedure.
6. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

Operation procedure

1. Numbering in advance, and mark B0, standard and sample. Recommend to read the OD value at the dual-wavelength 450/630 nm.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, store at 2-8℃, not frozen.
3. Solution preparation: dilute the concentrated washing buffer (10 × concentrated) and concentrated redissolving solution (10 × concentrated) to working solution.
4. Add 80 μL 0.0 ng/mL standard solution to B0 well.
5. Add 80 μL standard solution to each standard wells.
6. Add 80 μL sample to each sample wells.
7. Add 50 μL enzyme conjugate into every well(Recommend multi-channel Micropipettors), shake gently for seconds, then incubate at 20-25 ℃ for 40 min(often pat plate gently so as to reduce the error of duplicate wells).
8. Washing(3-4 times): Pour out the liquid, full washing buffer(250-300 μL) in all wells. repeat 3 times. flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
9. Coloration: after washing, add 50 μL of the substrate A solution and then 50 μL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 20-25 ℃ for 10-15 min.
10. Add 50 μL of the stop solution into each well, Mix gently by shaking the plate manually. Read the OD value at 450 nm within 5 min.