Antibiotics ELISA kits available:

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 us

Instruction Manual No. M-DE-100140

Ampicillin ELISA KIT

Cat. # DE-100140.

For Quantitative and Quantitative Determination of Ampicillin in pork liver, chicken, duck, shrimp, fish, honey and milk.

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LIFE TECH Life Technologies™
Your Molecular & Cell Technology Partner

Ampicillin ELISA KIT Cat. # DE-100140

Kit Components, 96 tests	Cat #
Micro-well strips: 12 strips with 8 removable wells each	DE100141
1× standard solution (5 mL) 8.1 ppb	DE100142
Enzyme conjugate (12 mL)	DE100143
Antibody working solution (7 mL)	DE100144
Substrate A solution (7 mL)	DE-SSA
Substrate B solution (7 mL)	DE-SSB
Stop solution (7 mL)	DE-ST
20× concentrated washing buffer (40 mL)	DE-WB
20× concentrated redissolving solution (50 mL)	DE-SS20
Instruction Manual	M-DE-100140

INTRODUCTION

Ampicillin is one of the types of antibiotics from the penicillin group that is used for treating bacterial infections. It is a white with slight yellow cast powder and is soluble in water. Its chemical formula is C16H19N304S-3H2O and its molecular weight is 349.406 g/mol. It has been used since 1961 to treat many bacterial infections in the body, including respiratory tract, urinary tract, gastrointestinal, some sexually transmitted diseases, and meningitis. It is also used to treat infections caused by E. coli and Salmonella.

It is a β-lactam antibiotic and has an amino group, which makes it different from penicillin. The amino group helps the ampicillin penetrates the wall membrane of Gram-positive and some Gram-negative bacteria. Ampicillin plays a role of being a competitive inhibitor of the enzyme transpeptidase. Transpeptidase is needed for the formation of the cell wall. It is an enzyme that catalyzes the transfer of an amino acid residue or a peptide residue from one amino compound to another. Ampicillin interferes with the third and final stage of the cell wall formation, which eventually leads to lysis of the cell. Therefore, ampicillin is most effective during cell division and forming cell walls.

Ampicillin is also used in research especially for bacterial transformation in molecular biology. It is usually used to help to confirm the uptake of the target genes by the Escherichia coli (E. coli). E. coli is constantly exchanging genetic information which is encoded in making proteins. Researchers take advantage of this mechanism to produce a variety of proteins. The gene of interest is added into the plasmid along with ampicillin and introduced to E. coli. E. coli obtains this gene combination with ampicillin and the gene of interest. Only the E. coli that successfully become ampicillin resistant will contain the target gene.

Alpha Diagnostic Intl's Ampicillin ELISA kit is a highly sensitive competitive type assay for the measurement of Ampicillin in pork liver, chicken, duck, shrimp, fish, honey and milk.

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 Ampicillin in the sample.

Qualitative determination

The concentration range (ng/mL) can be obtained from comparing the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample is 0.310, and that of the sample is 0.820, while those of the standard solutions are as the followings: 1.610 for 0 ppb, 1.350 for 0.1 ppb, 1.030 for 0.3 ppb, 0.660 for 0.90 ppb, 0.389 for 2.7 ppb and 0.198 for 8.1 ppb, accordingly the concentration range of the sample is 2.7 to 8.1 ppb, and that of the sample is 0.3 to 0.9 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 solutions and the semilogarithmic values of the Ampicillin standard solutions (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 actual concentration of Ampicillin 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 : 0.1 ppb

Detection limit:

Chicken, duck, pork, liver, fish, shrimp.....2 ppb
Honey, milk..... 2 ppb
Because of some interference in honey and milk, the detection limit is 4 ppb.

Recovery rate:

Chicken, duck, pork, liver, fish, shrimp..... 75%±10%
Honey, milk.....70%±10%

Cross-reaction rate:

Ampenicillin..... 100%
Benzyl penicillin.....0.8%
Cloxacillin..... 0.2%
Dicloxacillin.....0.1%
Amoxicillin..... 0.1%
Ceftiofu..... 0.1%

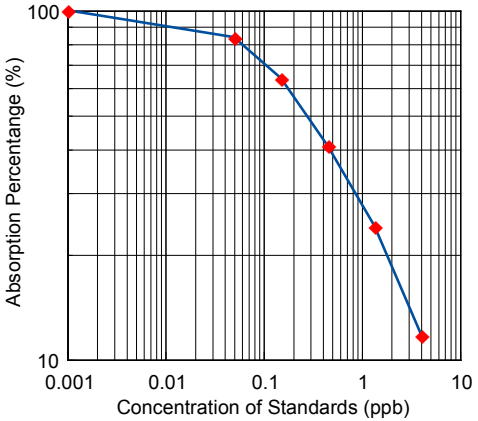
Precautions

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 standard solution 1 (0 ppb of less than 0.5 indicates its degeneration.
8. Coloration time is 15 min after the addition of the substrate A and then the B solution, if the color is light, prolong the time, don't exceed 30mi.
9. The optimum reaction temperature is 37 , and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values

Work Sheet of Typical Assay-Ampicillin

Wells	Stds/samples	Mean $A_{450\ nm}$	Absorption Percentage
A1, A2	**Standard A** 0.0 ppb	1.610	100%
B1, B2	**Standard B** 0.1 ppb	1.350	83.85%
C1, C2	**Standard C** 0.3 ppb	1.030	63.98%
D1, D2	**Standard D** 0.9 ppb	0.660	40.99%
E1, E2	**Standard E** 2.7 ppb	0.389	24.16%
F1, F2	**Standard F** 8.1 ppb	0.198	12.30%

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 Ampicillin in the sample. The antigens conjugated Ampicillin is pre-coated on the micro-well stripes, Ampicillin in the sample and the conjugated antigens pre-coated on the micro-well stripes compete for the anti-Ampicillin antibodies. 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 Ampicillin concentration in the sample. This value is compared to the standard curve and concentration of Ampicillin in the sample is subsequently obtained.

MATERIALS AND EQUIPMENT REQUIRED

Equipments: microplate reader, printer, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipettes, balance (a reciprocal sensibility of 0.01 g).
Micropipettes: single-channel 20-200 µL and 100 to 1000 µL, and multi-channel 250 µL.
Reagents: NaOH, Acetonitrile(CH_3CN), N-Hexane, deionized water, $Na_2Fe(CN)_5{\cdot}NO{\cdot}2H_2O$ and $ZnSO_4$.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Ampicillin ELISA 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

The following points must be dealt with before the pre-treatment of any kind of sample:
Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents.
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.

Solution preparation before sample pre-treatment:

1. 20×concentrated redissolving solution is mixed with deionized water at 1:19 (1 mL 20× concentrated redissolving solution+19 mL deionized water), used for sample redissolving.
2. 0.1M NaOH(for milk sample) : dissolve 0.4 g NaOH in deionized water to 100 mL.
3. CH3CN-0.1M NaOH solution: CH3CN: NaOH=84:16 (84 mL CH3CN + 16 mL 0.1 M NaOH).
4. C solution(for milk sample): dissolve 10.7 g Na2Fe(CN)5·NO·2H2O in deionized water to 100 mL
5. D solution(for milk sample): dissolve 28.8 g ZnSO4·7H2O in deionized water to 100 mL.

Tissue (chicken, duck, pork, liver, fish, or shrimp)

1. Homogenize the sample.
2. Take 2± 0.05 g of the homogenized sample into 50 mL centrifugal tube, add 2 mL 1 M NaOH solution, rock it with vortex for 2 min, add 8 mL CH_3CN, shake for 10 min, centrifuge at above 4000 r/min at room temperature (20-25) for 10 min.
3. Take 1 mL of the supernatant, blow to dry by nitrogen or air at 50-60 .
4. Dissolve the dry residues in 1 mL of the diluted redissolving solution, then dilute at 1:3 (50 µL sample + 150 µL the diluted redissolving solution).
5. Take 50 µL for further analysis.

Fold of dilution of the sample: 20

Honey

1. Put 1.0 ± 0.05 g honey into centrifuge tube, add 3 mL diluted redissolving solution, then static for 20 min after shaking vigorously. centrifuge at above 4000 r/min at room temperature (20-25) for 10 min.
2. Take upper layer, diluted 1:4 in diluted redissolving solution (20 µL sample + 80 µL the diluted redissolving solution).
3. Take 50 µL for further analysis.

Fold of dilution of the sample: 20

Milk

Method A

1. Take 2 mL fresh milk into 5 mL centrifugal tube, add 50 µL C solution, mix properly.
2. Add 50 µL D solution ,shake for 1 min, centrifuge at 4000 r/min at 10 for 10 min.
3. Take clear liquid(upper layer), dilute with the diluted redissolving solution at 1:19 (20 µL sample+380 µL diluted redissolving solution).
4. Take 50 µL for further analysis.

Fold of dilution of the sample: 20

Note: Repeat again if centrifuged sample is muddy

Method B

1. Put 2 mL milk (removed fat) into centrifugal tube.
2. Add 8 mL of the CH_3CN-0.1 M NaOH solution, shake vigorously for 10min, centrifuge at above 4000 r/min at 15 ℃ for 10 min, take 1 mL supernatant (upper layer), evaporate to dryness by nitrogen at 60 ℃
3. Dissolve the dry residues in 1 mL N-Hexane, add 1 mL of the diluted redissolving solution, mix properly for 1 min, centrifuge and remove N-hexane phase.
4. Take the lower to dilute 1:3 (50 µL sample + 150 µL the diluted redissolving solution).
5. Take 50 µL for analysis.

Fold of dilution of the sample: 20

STORAGE AND STABILITY

Storage: store at 2-8 ℃, not frozen.

Expiration date: 12 months; please check date of production before use.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Instructions

1. Bring all reagents and micro-well strips to the room temperature (20-25 ℃).
2. Return all reagents to 2-8 ℃ immediately after use.
3. 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 the procedures of ELISA.
4. 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. Take out the kit from 4 ℃ environment. Take out all the necessary reagents from the kit and place at the room temperature (20-25 ℃) for at least 30 min. Note that each reagent must be shaken to mix evenly before use.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.
3. Solution preparation: dilute 40 mL of the concentrated washing buffer (20×concentrated) with the distilled or deionized water to 800 mL (or just to the required volume) for use.
4. Prepare for standard solution:
   2.7ppb standard solution: Dilute 100 µL standard solution(8.1 ppb) with 200 µL the diluted redissolving solution.
   0.9 ppb standard solution: Dilute 100 µL standard solution(2.7 ppb) with 200 µL the diluted redissolving solution.
   0.3 ppb standard solution: Dilute 100 µL standard solution(0.9 ppb) with 200 µL the diluted redissolving solution.
   0.1 ppb standard solution: Dilute 100 µL standard solution(0.3 ppb) with 200 µL the diluted redissolving solution.
   0 ppb standard solution: 200 µL the diluted redissolving solution.
5. Numbering: number the micro-wells according to samples and standard preparation; each sample and standard solution should be performed in duplicate; record their positions.
6. Add 50 µL of the sample or standard solution to separate duplicate wells, and add 50 µL of antibody working solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min.
7. Pour the liquid out of the wells ,wash the microplate with the washing buffer at 250 µL/well for 4-5 times. Each time soak the well with the washing buffer for 10 s and then flap to dry on absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
8. Add 100 µL of the enzyme conjugate into every well, seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min, continue as subscribed in step 6.
9. Coloration: 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 37 ℃ for 15 min at dark for coloration.
10. Determination: add 50 µL stop solution into each well. Vortex evenly. Set the wavelength of the microplate reader at 450 nm to determine the OD value (we recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).