Antibiotics ELISA kits available from ADI:

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

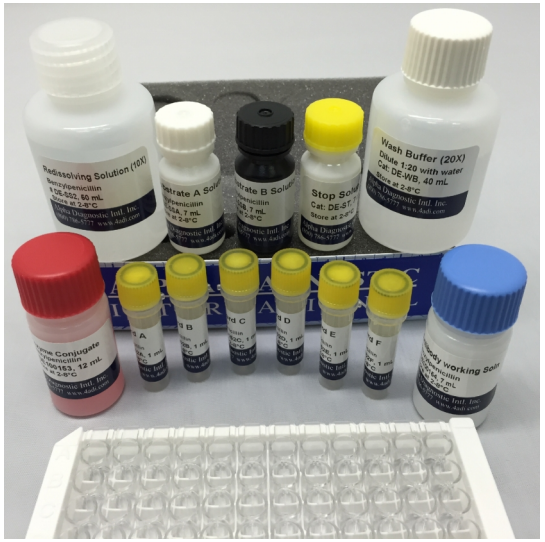
LIFE TECH Life Technologies™
Your Molecular & Cell Technology Partner

adi

Instruction Manual No. M-DE-100150

Benzyl penicillin ELISA KIT

Cat. #DE-100150



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

Kit Components, 96 tests	Cat #
Micro-well **coated strip plate** (12 strips with 8 removable wells each)	DE-100151
6x standard solution (1ml each): 0.0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb	DE-100152
Enzyme conjugate (12ml)	DE-100153
Antibody working solution (7ml)	DE-100154
Substrate A solution (7ml)	DE-SSA
Substrate B solution (7ml)	DE-SSB
Stop solution (7ml)	DE-ST
20X Concentrated Washing buffer (40ml)	DE-WB
10X Concentrated redissolving solution (50ml)	DE-SS2
Instruction Manual	M-DE-100150

INTRODUCTION

Benzylpenicillin is also known as penicillin G. Its chemical formula is C16H18N2O4S and its molecular weight is 334.4 g/mol. Penicillin history started with the Scottish scientist Alexander Fleming in 1928. The Scottish scientist showed that if the organism Penicillium notatum was grown in the appropriate substrate it would derive an antibiotic substance.
Benzylpenicillin is usually used on the treatment of cellulitis, bacterial endocarditis, gonorrhea, meningitis, aspiration pneumonia, syphilis, septicemia and septic arthritis. Benzylpenicillin can not be given orally since it is very unstable in the hydrochloric acid of the stomach. Since it is given by injection or infusion benzylpenicillin can reach to a very high concentration on the tissue leading to increase of antibacterial activity. The uptake of benzylpenicillin can lead to the following side effects rash, itching, diarrhea, fever, or inflammation of the large intestine.

All penicillins are Beta-lactam antibiotics which in clinical use are the main active antibiotics against Gram-positive bacteria. Beta-lactam antibiotics are bactericidal, which act by inhibiting the synthesis of the peptidoglycan layer of the wall on the bacteria. Peptidoglycan is formed of sugars and amino acids that form the layer outside the plasma membrane of the bacteria cell wall. Penicillin interferes with the production of peptidoglycan by binding to bacterial enzymes known as transpeptidases.

Benzylpenicillin is also used in the treatment of animal disease to promote a healthy environment for the farming industry. However if the animals are treated with high doses of benzylpenicillin, its residues can cause allergic reaction and tolerance to the drug. Benzylpenicillin was banned in the EU and America.

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

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 Benzylpenicillin 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 Ⅰis 0.310, and that of the sample Ⅱ 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 Ⅰis 2.7 to 8.1 ppb, and that of the sample Ⅱ is 0.30 to 0.90 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 Benzylpenicillin 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, finally obtaining the Benzylpenicillin 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: 0.1 ppb
Detection limit
Chicken, duck, pork, liver, fish, shrimp..... 2 ppb
Honey, milk..... 2 ppb
Recovery rate
Chicken, duck, pork, liver, fish, shrimp.....85±10%
Honey, milk.....70±10%
Cross-reaction rate
Benzyl penicillin.....100%
Ampicillin..... 0.8%
Cloxacillin..... 0.2%
Dicloxacillin.....0.1%
Amoxicillin..... 0.1%
Ceftiofur..... 0.1%

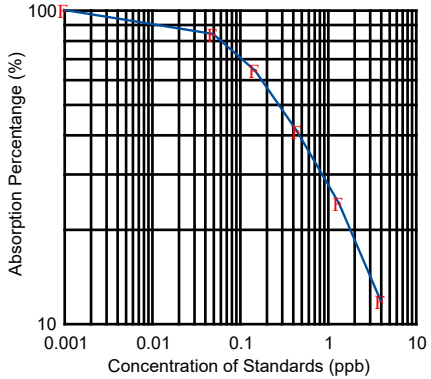
NOTES:

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 30min.
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-Benzylpenicillin

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 Benzylpenicillin in the chicken, pork, duck, honey, milk, fish, shrimp and egg, etc. The antigens conjugated Benzylpenicillin is pre-coated on the micro-well stripes, Benzylpenicillin in the sample and the conjugated antigens pre-coated on the micro-well stripes compete for the anti-Benzylpenicillin 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 Benzylpenicillin concentration in the sample. This value is compared to the standard curve and concentration of Benzylpenicillin 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)
Micropipettors: single-channel 20 to 200 µL and 100 to 1000 µL, and multi-channel 250 µL;
Reagents: NaOH,Acetonitrile (CH3CN), N-Hexane, deionized water, Na2Fe(CN)5·NO·2H2O and ZnSO4·7H2O, HCl (36%), 2M H2SO4

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Benzylpenicillin 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.

Solution preparation before sample pre-treatment
1. **Sample diluent**: The 10X concentrated redissolving solution is mixed with deionized water at **1:9** (1 mL concentrated redissolving solution + 9 mL deionized water), used for sample redissolving.
2. 0.1 M NaOH (for milk sample) :dissolve 0.4 g NaOH in deionized water to 100 mL.
3. CH3CN-0.1 M NaOH solution: VCH3CN : VNaOH =84:16 (84 mL CH3CN + 16 mL 0.1 M NaOH).
4. C solution (for milk sample)/0.36 M Na2Fe(CN)5·NO·2H2O:dissolve 10.7 g Na2Fe(CN)5·NO·2H2O in deionized water to 100 mL .
5. D solution (for milk sample)/1 M ZnSO4 : dissolve 28.8 g ZnSO4·7H2O in deionized water to 100 mL.
6. Acidic Acetonitrile (CH3CN) (for honey sample): add 150 µL 2 M H2SO4 into 100 mL Acetonitrile (CH3CN),mix properly.

7. 1 M HCl (for honey sample): dissolve 41.7 mL HCl (36%) in deionized water to 500 mL.
8. 1 M NaOH (for honey sample): dissolve 4 g NaOH in deionized water to 100 mL.

Samples preparation

a) Tissue (chicken, duck, fish, shrimp, pork or pork liver)
1. Homogenize the sample.
2. Take **1± 0.05 g** of the homogenized tissue sample into 50 mL centrifuge tube, add 2 ml 0.2M HCl solution, vortex for 3 min;
3. Then add **400ul** 1M NaOH solution and **1.6 ml** Sample diluent, shake for 3 min, centrifuge at above 4000 r/min at room temperature(20-25 ℃) for 5 min;

3) Take the supernatant, dilute with Sample diluent at **1:2** (**50u**l supernatant + **100ul** Sample diluent), mix for 30 seconds.

4) Take **50 µL** for analysis.

Fold of dilution of the sample: 15

b) Honey:

1 Put 1.0 ± 0.05 g honey into centrifuge tube , add 3 mL diluted redissolving solution, mix properly, then place it for 20 min, centrifuge at above 4000 r/min at room temperature (20-25℃) for 10 min.

2 Taker the supernatant (upper layer), dilute with redissolving solution at 1:4 (**20 µL** sample+**80 µL** diluted redissolving solution), mix for 30s.

3 Take **50 µL** for further analysis.

Fold of dilution of the sample: 20

c) Milk
Method A
1. Take 2 mL fresh milk into 5 mL centrifuge 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 centrifuge tube.
2. Add 8 mL of the CH_3CN-0.1 M NaOH solution, shake vigorously for 10 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min, take 1 mL of the clear liquid (upper layer) and 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 to 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 FOR AT LEAST 30 min)).*

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. Numbering: number the micro-wells according to samples and standard preparation; each testing sample and standard solution should be performed in duplicate; record their positions.
5. Add **50 µL** of the sample or standard solution to separate duplicate wells, and add **50 µL** of the antibody working solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at **25 ℃ for 30 min**.
6. 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).
7. Add **100 µL** of the enzyme conjugate into every well, seal the microplate with the cover membrane, and incubate at **25 ℃** for **30 min**, continue as subscribed in 6.
8. 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 **25 ℃** for **15 min** at dark for coloration.
9. 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).

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Road No. 44, Pitampura, Delhi – 110034, India
Mobile: +91-98105-21400, Tel: +91-11-42208000, 8111, 8222, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com, www.atzlabs.com ; www.lifetechindia.com