Penicillin G(benzyl penicillin) ELISA Kit

Catalog Number. CSB-E12095f

This immunoassay kit allows for the in vitro quantitative determination of benzyl penicillin concentrations in tissue(meat, liver, fish, shrimp), honey, milk, milk powder, ice cream, cream and other samples.

This package insert must be read in its entirety before using this product.

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: www.cusabio.com

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique.
The coupling antigen is pre-coated on the micro-well stripes. The Benzyl penicillin in the testing sample competes with the coupling antigens pre-coated on the micro-well stripes for the antibodies against Benzyl penicillin. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the testing sample has a negative correlation with the content of Benzyl penicillin in it. This value is compared to the standard curve and the content of the corresponding Benzyl penicillin is subsequently obtained.

DETECTION RANGE

0.1 ppb-8.1 ppb.

SENSITIVITY

The minimum detectable dose of Benzyl penicillin is typically less than 0.1 ppb.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest concentration that could be differentiated from zero. It was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations.

Detection limit

tissue	2ppb
honey	0.1ppb
milk	2ppb
milk powder	4ppb
ice cream, cream	1ppb

Recovery rate

tissue	85% ±10%
honey	70% ±10%
milk, milk powder, ice cream, cream	85% ± 25%

Cross-reaction rate

Benzyl penicillin	100%
Ampicillin	0.7%
Cloxacillin	0.2%
Dicloxacillin	0.1%
Amoxicillin	＜0.1%
Ceftiofur	＜0.1%

PRECISION

Intra-assay Precision (Precision within an assay): CV%<10%
Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<10%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate (96 tests)	1
Standard	6 x 1 ml
HRP-conjugate	1x 12 ml
Antibody	1 x 7 ml
Substrate A	1x 7 ml
Substrate B	1x 7 ml
Stop Solution	1x 7 ml
Wash Buffer(20×concentrate)	1 x 40 ml
Redissolving Solution(2×concentrate)	1 x 50 ml

STANDARD CONCENTRATION

Standard	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1

STORAGE

Unopened kit	Store at 2 - 8°C. Do not use the kit beyond the expiration date.
Opened kit	May be stored for up to one month at 2 - 8° C.

***Provided this is within the expiration date of the kit.**

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100 ml and 500 ml graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.
- NaOH, acetonitrile, n-hexane, concentrated hydrochloric acid, concentrated sulfuric acid

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Note:

- **Kindly use graduated containers to prepare the reagent.**
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

REAGENT PREPARATION

1. **0.1M NaOH Solution**: dissolve 0.4 g NaOH in deionized water to 100 ml.
2. **Acetonitrile/0.1M NaOH mixed solution**: 84ml Acetonitrile and 16ml 0.1M NaOH, mixed well.
3. **Acidified acetonitrile solution**: take 100ml acetonitrile and mix with 150μl 2M sulfuric acid.
4. **1M hydrochloric acid solution**: dilute 41.5ml concentrated hydrochloric acid with deionized water, and dilute to 500ml.
5. **1M NaOH Solution**: dissolve 4 g NaOH in deionized water to 100 ml.
6. **Redissolving Solution**: the 2x concentrate Redissolving Solution is diluted with deionized water at 1:1 (eg:1 ml concentrated Redissolving Solution + 1 ml deionized water).
7. **Wash Buffer**: If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of 20x concentrate Wash Buffer with deionized or distilled water to prepare 400 ml of Wash Buffer.

Note:

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
4. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
5. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

SAMPLE COLLECTION AND STORAGE

A: Tissues(chicken, duck, pork, liver, shrimp, fish)

(1) Homogenize the sample at 10000 r/min for 1 min.
(2) Weigh 2.0±0.05 g of the homogenized sample, put into 50ml centrifugal tube, add 8ml Acetonitrile/0.1M NaOH mixed solution. Vortex for 2min with a vortex meter. And shake properly with oscillator for 20min.
(3) Centrifuge at more than 3000g for 10 min at room temperature.
(4) Transfer 1ml supernatant into a new centrifugal tube, evaporated to dryness at 50-60°C by nitrogen flow nitrogen in water bath.
(5) Add 1ml of the N-hexane to redissolve drying residue. Then add 1ml diluted redissolving solution and shake strongly for 30s. Centrifuge at more than 3000g at room temperature for 5 min.
(6) Remove the upper layer. Dilute 50μl of lower into 200μl of diluted redissolving solution(1:4).
(7) Take 50μl for further analysis. Fold of dilution of the samples: 20

B: Honey

(1) Weight 4±0.05 g honey into 50ml centrifugal tube, then add 0.5 ml 1 M NaOH. Mix well and then be static for 20 min.
(2) Add 0.5 ml 1 M HCl (adjust pH to 3), mix well. Then add 7 ml Acidification acetonitrile (adjust pH to 4.0), shake for 5 min, centrifuge at above 3000 g at room temperature for 10 min.
(3) Take 3 ml supernatant, evaporated to dryness at 50-60°C by nitrogen flow nitrogen in water bath..
(4) Add 1 ml of the diluted redissolving solution, redissolve it for 1 min.
(5) Take 50μl for further analysis. Fold of dilution of the sample: 1

C: Fresh milk

(1) Take 20μl fresh milk into 2ml centrifugal tube. Add 380μl of diluted redissolving solution. Shake for 1min.
(2) Take 50μl for further analysis. Fold of dilution of the sample: 20

D: Milk powder

(1) Weight 1.0±0.05g milk powder into 50ml centrifugal tube.
(2) Add 5 ml ddH_2O. Mix well with oscillator.
(3) Dilute 50μl sample into 350μl of diluted redissolving solution, mix well.
(4) Take 50μl for further analysis. Fold of dilution of the samples: 40

E: Ice cream and cream

(1) Weight 1.0±0.05g ice cream or cream samples into 50ml centrifugal tube.
(2) Add 1 ml methanol. Mix well with oscillator.
(3) Then add 4ml diluted redissolving solution and shake properly for 5s.
(4) Dilute 200μl sample into 200μl of diluted redissolving solution, shake properly for 5s.
(5) Take 50μl for further analysis. Fold of dilution of the samples: 10

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Add 50µl of **Standard** or **Sample** per well. Standard and Samples need test in duplicate.
4. Add 50µl of **Antibody** to each well. Mix well and then incubate for 30 min at 37°C.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with **Wash Buffer** (250µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 10 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash Buffer by aspirating ordecanting. Invert the plate and blot it against clean paper towels.
6. Add 100µl of **HRP-conjugate** to each well. Mix well and then incubate for 30 min at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
7. Repeat the aspiration/wash process for five times as step 5.
8. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well, mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
9. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 10 minutes). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the Substrates. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrates.

CALCULATION OF RESULTS

Using the professional soft "Curve Expert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

(1) The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbency value}(\%) = \frac{B}{B_0} \times 100\%$$

B ——absorbance standard (or sample)
B0 ——absorbance zero standard

(2) To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the Benzyl penicillin standards solution (ppb) as x-axis.

The Benzyl penicillin concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.