Penicillin G (benzyl penicillin) ELISA kit

Cat#: orb411521 (ELISA Manual)

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 (LOD) 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.

LIMIT OF DETECTION

Tissue	2ppb

RECOVERY RATE

Tissue	60%~120%

CROSS-REACTION RATE

Benzyl penicillin	100%
Ampicillin	0.8%
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

- 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(96T)
Assay plate (96 tests)	96T
Standard	6 x 1 mL
HRP-conjugate	1 x7 mL
Antibody	1 x 7 mL
Substrate A	1 x 7mL
Substrate B	1 x 7mL
Stop Solution	1 x 7mL
Wash Buffer (20×)	1 x 40 mL
Redissolving Solution (10×)	1 x 50 mL
Adhesive Strip	4
Instruction Manual	1

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 1 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 25°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Centrifuge, Vortex mixer.
- Analytical balance, 2 decimal places
- Single-channel micropipette (20 µL-200 µL、100 µL-1000 µL).
- 300 µL multichannel micropipette.
- 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.
- Concentrated HCl.

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 (20-25°C) before use for 30 min.
- Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents.
- 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

- **0.2M HCl:** Transfer 17.2 mL of **Concentrated HCl** to 1000 mL of deionized water, shake well.
- **1M NaOH**: Dissolve 4g NaOH to 100 mL of deionized water, shake well.
- **Sample Diluent**: The **Redissolving Solution (10x)** is diluted with deionized water at 1:9 (eg:1 mL Redissolving Solution + 9 mL deionized wate, shake wellr).

- **Wash Buffer**: If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 40 mL of **Wash Buffer (20x)** with deionized or distilled water to prepare 800 mL of **Wash Buffer (1x)**. Keep it at 4℃ for one month.

Note:

1. Biorbyt 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.

SAMPLE COLLECTION

Tissue (pork, liver, chicken)

1. Weigh 1.00±0.05g of the homogenized tissue sample.
2. Add 2mLof**0.2M HCl,** shake properly for 3 min.
3. Add 400 µL of **1M NaOH** and 1.6 mL of **Sample Diluent**, shake properly for 3 min.
4. Centrifuge at more than 4000 r/min for 5 min at room temperature.
5. Transfer supernatant into a new centrifugal tube, add **Sample Diluent** (eg: 50 µL of supernatant +100 µL of **Sample Diluent**), shake properly for 30 s.
6. Take 50 µL of sample for further analysis.

Dilution factor of the samples: 15

ASSAY PROCEDURE

Bring all reagents and samples to room temperature (20~25℃) 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 2-8°C.
3. Add 50 µL of **Standard** or **Sample** per well. Then add 50 µLof **Antibody** to each well. Cover the microtiter plate with a new adhesive strip and mix well, then incubate for 30 min at 25°C.
4. Aspirate each well and wash, repeating the process 4~5 times. Wash by filling each well with 250 µLof **Wash Buffer (1x)** using a squirt bottle, multi-channel pipette, manifold dispenser, or auto washer and let it stand for 15~30 seconds, complete removal of liquid at each step is essential to good performance.
5. Add 100 µLof **HRP-conjugate** to each well. Mix well and then incubate for 30 min at 25°C.
6. Repeat the wash process as step 4.

7. Add 50 µL of **Substrate A** and 50 µL of **Substrate B** to each well, mix well. Incubate for 15 minutes at 25°C. Protect from light.
8. Add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 5 min, using a microplate reader set to 450 nm (Recommend to read the OD value at the dual-wavelength: 450/630 nm within 5 min).

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 min. 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 15~30 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 min). 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.