Penicillin ELISA Kit

Enzyme Immunoassay for the quantitative determination of Penicillin in milk and shrimps

Catalog number: ARG80810

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

Penicillin was accidentally detected by Alexander Fleming in 1929. The drug belongs to the mycotoxins and is generated by the mold Penicillium chrysogenum. Penicillin as an antibiotic is preferentially used in the treatment of gram-positive bacteria, both for humans and animals. Of all illegally administered drugs, antibiotics are most frequently used in produc-tive livestock. Contaminations in food or milk are in-gested by humans, and can lead to severe infections by pathogen germs which became resistant against penicillin, or to allergies. The allergic reactions ap-pear with different severity, dependent on dose and individual disposition, and showing symptoms from urticaria to anaphylactic shock.

During routine testing of milk samples for antibiotics, in more than 90% of the positive cases, beta-lactam preparations or penicillins are detected.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative competitive enzyme immunoassay technique. A penicillin conjugate is bound on the surface of a microtiter plate. Penicillin containing samples or standards and an antibody directed against penicillin are given into the wells of the microtiter plate. Immobilized and free penicillin compete for the antibody binding sites. After one hour incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugate directed against the penicillin antibody is given into the wells and after another hour incubation, the plate is washed again. Then a substrate solution is added and incubated for

20 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of penicillin is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Penicillin-coated microplate	12 strips x 8-well	4°C
Anti-Penicillin Antibody	6 ml (ready to use)	4°C
HRP-conjugated antibody (HRP-anti-mouse-IgG)	15 ml (ready to use)	4°C
Standard A-F (0, 4, 10, 40, 100, 400 ng/ml)	6 X 1 ml (ready to use)	4°C
10X Sample Diluent	2 X 50 ml	4°C
10X Wash Buffer	60 ml	4°C
TMB substrate	15 ml (ready to use)	4°C (Protect from light)
STOP solution	15 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times. Do not use reagents after expiration date.
- Briefly spin down the anti-penicillin antibody and HRP-conjugated antibody before use.
- If crystals are observed in the 10X Wash buffer and 10X sample diluent, warm to RT or 37°C for 15min or until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- All specimens and standards should be run at the same time, so that all conditions of testing are the same.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer. (E.g. 50 ml of 10X Wash buffer + 450 ml of distilled water).
- 1X sample diluent: Dilute 10X sample diluent into distilled water to yield 1X sample diluent. (E.g. 50 ml of 10X sample diluent + 450 ml of distilled water).

SAMPLE COLLECTION & STORAGE INFORMATION

Shrimps

- Homogenize sample with ultra-turrax or mixer.
- Add **1 g** homogenized sample to **4 mL** of diluted **1X sample diluent** in a glass vial and shake heavily for **20 minutes**.
- Centrifuge sample afterwards for **10 minutes at 3000 g**.
- Dilute supernatant **1:5** in diluted 1X sample diluent. This solution can now be directly inserted in the ELISA.

Milk

- Pipet 5 mL of a fresh milk sample (full-cream milk or skim milk) into a glass vial and incubate for 30 minutes at 2-8°C.
- Centrifuge sample afterwards for 10 minutes at 3000 g.
- Separate the upper fat layer and dilute milk 1:4 in diluted 1X sample diluent. This solution can now be directly inserted in the ELISA.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards and samples** in duplicate into the appropriate wells of the microtiter plate. Immediately add **50 µl** of **penicillin antibody** into each well.
3. Cover the microtiter plate with a plastic foil and incubate for **60 minutes** at **room temperature** on a microtiter plate shaker (or 90 minutes without shaker).
4. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes.** Wash by filling each well with **1X wash buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Pipet **100 µL** of **HRP-conjugated antibody** (HRP-anti-mouse-IgG) into each well.
6. Cover the microtiter plate with a plastic foil and incubate for **60 minutes at room temperature** on a microtiter plate shaker (or 90 minutes without shaker).
7. **Wash** the wells as **step 4.**
8. Add **100 µl** of **TMB substrate mixture** to each well. Incubate for **20 minutes**

at room temperature in dark.

9. Add 100 µl of Stop Solution to each well.
10. Read the OD with a microplate reader at 450 nm immediately (optional: reference wavelength at 620 nm). It is recommended reading the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

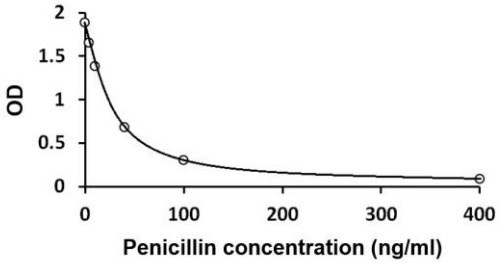
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. The diluted samples must be further converted by the appropriate dilution factor. The dilution factor is 20 for shrimps and 4 for milk extraction according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The sensitivity of the Penicillin ELISA is 3 ng/mL (based on the standard curve).

Intra-assay

The intra-assay variation of the penicillin test was determined to 3 %.

Recovery

The recovery of spiked samples was determined to 91-102% for milk and 62-71 % for shrimps.

Cross Reactivity

Various beta-lactam antibiotics were tested with Penicillin ELISA test kit. The cross-reactivity % were at the below table.

Substance	Mean Cross Reactivity (%)
Penicillin V	3.1
Ampicillin	0.4
Amoxicillin	0
Cephalexin	0.03
Cloxacillin	0.02