



## Test Instruction

Penicillin

96 Tests

Enzyme Immunoassay  
for the Quantitative  
Determination of  
Penicillin in Milk and Shrimps

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**Gold Standard Diagnostics  
Kassel GmbH**

Otto-Hahn-Str. 16  
D-34123 Kassel  
Germany

Phone: +49 (0) 561 491742-0  
Fax: +49 (0) 561 491742-20

[info.kassel@eu.goldstandarddiagnostics.com](mailto:info.kassel@eu.goldstandarddiagnostics.com)

[www.kassel.goldstandarddiagnostics.com](http://www.kassel.goldstandarddiagnostics.com)

Sensitivity	3 ng/mL
Recovery (milk)	90%
Recovery (shrimp)	70%
Incubation Time	140 min

## General Information

Penicillin was accidentally detected by Alexander Fleming in 1929. The drug belongs to the mycotoxins and is generated by the mould *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 productive livestock. Contaminations in food or milk are ingested by humans, and can lead to severe infections by pathogen germs which became resistant against penicillin, or to allergies. The allergic reactions appear 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, betalactam preparations or penicillins are detected.

The method of choice for the determination of penicillin contamination in food has always been a microbiological assay. These procedures allow however no quantitative determination and no identification of the antibiotic drug, which is achieved by a sensitive ELISA test kit or immunoaffinity columns together with HPLC.

## Principle of the Test

The **Gold Standard Diagnostics Penicillin** quantitative test is based on the principle of the enzyme linked immunosorbent assay. 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 colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of penicillin is indirectly proportional to the colour intensity of the test sample.

## Precautions

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micro-pipets, ELISA reader etc.).

## Health and safety instructions

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

## Reagents

The kit contains reagents for 96 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with penicillin conjugate.

2. Penicillin Standards (0; 4; 10; 40; 100; 400 ng/mL): 6 vials with 1.0 mL each, ready-to-use.
3. Anti-Penicillin Antibody (mouse): 6 mL, dyed red, ready-to-use.
4. Conjugate (anti-mouse-IgG-HRP): 15 mL, dyed red, ready-to-use.
5. Substrate Solution (TMB): 15 mL; ready-to-use.
6. Stop Solution (0.5 M H<sub>2</sub>SO<sub>4</sub>): 15 mL; ready-to-use.
7. Sample Diluent (PBS): 2 x 50 mL as 10x concentrate. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
8. Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate, dyed blue. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.
9. Two plastic foils to cover the strips during the incubation.
10. Plastic bag to store unused microtiter strips.
11. Instruction Manual.

## Additional Instrumentation and Reagents (not provided)

### *Instrumentation*

- 50, 100 and 1000 µL-micropipets
- Microtiter plate shaker
- ELISA reader (450 nm)
- Ultra-turrax or mixer
- Centrifuge

## Sample Preparation

### *Shrimps*

- Homogenize sample with ultra-turrax or mixer.
- Add to **1 g** homogenized sample **4 mL diluted** 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 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 sample diluent. This solution can now be directly inserted in the ELISA.

## Procedure

1. Prepare samples as described above.
2. Pipet 100 µL ready-to use standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50 µL 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. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.
5. Pipet 100 µL of conjugate (anti-mouse-IgG-HRP) 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 plate as outlined in 4.
8. Pipet 100 µL of substrate solution into each well.
9. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
10. Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H<sub>2</sub>SO<sub>4</sub>) into each well. The blue colour will turn yellow upon addition.
11. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

## Calculation of results

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.

2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.
3. Using the mean optical density value for each sample, determine the corresponding concentration of penicillin in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. 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.

## Typical Standard Values

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in every new test.

Penicillin (ng/mL)	(% binding of 0 ng/mL)
0	100
4	85
10	70
40	35
100	15
400	5

## Performance

### Sensitivity

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

### Recovery

The recovery of spiked samples was determined to 90% for milk and 70% for shrimps.

### Intra-assay Precision

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

## References

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