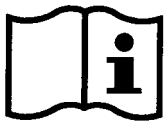
Product information

Information about other products is available at: www.demeditec.com



Useŕs Manual

Penicillin ELISA

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

REF **DEPENE01**

Σ **96 wells**

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • 24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
info@demeditec.de • www.demeditec.com

Version 170331
DMC
Updated 210119

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

1. GENERAL INFORMATION

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.

This test is designed for detection of substances within food products. This is not a medical device and is not intended to diagnose or prevent any diseases or other conditions.

2. PRINCIPLE OF THE TEST

The **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.

3. 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 (micropipets, ELISA reader etc.).

4. 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).

5. 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. **SORB MT** Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with penicillin conjugate.
2. **CAL 1 – 6** Penicillin Standards (0; 4; 10; 40; 100; 400 ng/mL): 6 vials with 1.0 mL each, ready-to-use.
3. **Ab** Anti-Penicillin Antibody (mouse): 6 mL, dyed red, ready-to-use.
4. **ENZ CONJ** Conjugate (anti-mouse-IgG-HRP): 15 mL, dyed red, ready-to-use.
5. **SUB TMB** Substrate Solution (TMB): 15 mL; ready-to-use.
6. **STOP SOLN** Stop Solution (1 N acidic solution): 15 mL; ready-to-use.
7. **SAM DIL 10x** 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. **WASH SOLN 10x** 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. Instruction Manual.

6. 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
- Plastic bag to store unused microtiter strips.

7. 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.

8. 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 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 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 (1 N acidic solution) 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.

9. 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** und **4 for milk** extraction according to the sample preparation procedure as described above.

10. 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

11. PERFORMANCE

Sensitivity
The sensitivity of the **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 %.

12. REFERENCES

1. De Leuw P, Kapa G, Petz M, Schreurs FJ, Kan CA; J AOAC Int. 80(6): 1220 (1997). Induction and characterization of multianalyte antibodies against penicillins in egg yolk.
2. Knecht BG, Strasser A, Dietrich R, Martlbauer E, Niessner R, Weller MG; Anal Chem. 76(3): 646 (2004). Automated microarray system for the simultaneous detection of antibiotics in milk.
3. Althaus RL, Molina MP, Rodriguez M, Fernandez N; J Food Prot. 64(11): 1844 (2001). Detection limits of beta-lactam antibiotics in ewe milk by penzym enzymatic test.
4. Huth SP, Warholic PS, Devou JM, Chaney LK, Clark GH; J AOAC Int. 85(2): 355 (2002). Parallux beta-lactam: a capillary-based fluorescent immunoassay for the determination of penicillin-G, ampicillin, amoxicillin, cloxacillin, cephapirin, and ceftiofur in bovine milk.
5. Martlbauer E, Usleber E, Schneider E, Dietrich R; Analyst. 119(12): 2543 (1994). Immunochemical detection of antibiotics and sulfonamides.
6. Usleber E, Lorber M, Straka M, Terplan G, Martlbauer E; Analyst. 119(12): 2765 (1994). Enzyme immunoassay for the detection of isoxazolyl penicillin antibiotics in milk.
7. Gustavsson E, Sternesjo A; J AOAC Int. 87(3): 614 (2004). Biosensor analysis of beta-lactams in milk: comparison with microbiological, immunological, and receptor-based screening methods.

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instruc-tions d'utilisation	Consulte las Instruc-ciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precau-ciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore

Distributed By:
IBL-America, Inc.
8201 Central Ave NE, Suite P
Minneapolis, MN 55432, USA
info@ibl-america.com
(888) 523 1246