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GENERAL INFORMATION

Product Description

MaxSignal® Penicillin ELISA Kit is a competitive enzyme immunoassay for the quantitative analysis of Penicillin and other beta-lactams such as ampicillin, amoxicillin, cloxacillin, and oxacillin. Penicillin is among the most widely used antibiotics for the treatment of bacterial infections in man and animals. Penicillin is also administered to animals in feed for growth promotion and for collective prophylactic treatment. The monitoring of penicillin residues in edible tissues and milk is important because of the hypersensitivity of some individuals to these antibiotics and also the emergence of antibiotic-resistant strains of bacteria. For this reason, maximum residue limits have been specified for food products and milk to try and control the levels of these antibiotics reaching the consumer.

MaxSignal® Penicillin ELISA Kit enables international and government regulatory agencies, food manufacturers and processors, as well as quality assurance organizations, to detect penicillin containing antibiotics to satisfy customer concerns about food safety.

Procedure Overview

The method is based on a competitive, two-step colorimetric enzyme-linked immunosorbent assay (ELISA). The target analyte has been coated to the plate wells. During analysis, sample is added along with a primary antibody specific to the target analyte. If the target analyte is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the analyte coated to the plate well. A secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the analyte coated on the plate wells. The resulting color intensity, after addition of TMB substrate, has an inverse relationship to the target analyte concentration in the sample.

Kit Contents, Storage and Shelf Life

MaxSignal® Penicillin ELISA Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (assuming 12 wells for standards). Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package. Store the entire kit at 2–8°C. Do not use this product past the expiration date indicated on the Certificate of Analysis.

Kit Contents	Amount	Storage
Penicillin-Coated Plate	1 x 96-well Plate (8 wells x 12 strips)	2-8 °C
Penicillin G powder stock (1,000 ng):	2	-20 °C
Empty Vials for Standards: Negative control (white cap tube) 0.08 ng/mL (yellow cap tube) 0.2 ng/mL (orange cap tube) 0.4 ng/mL (pink cap tube) 0.8 ng/mL (purple cap tube) 1.2 ng/mL (blue cap tube)	 1 1 1 1 1 1	
20X Wash Solution	28 mL	2-8 °C
10X Penicillin Capture Protein	1.8 mL	2-8 °C
Penicillin Capture Protein Diluent	12 mL	2-8 °C
Antibody #2 Diluent	20 mL	2-8 °C
200X HRP-Conjugated Antibody #2	100 µL	2-8 °C
TMB Substrate	12 mL	2-8 °C
Stop Solution	14 mL	2-8 °C
Milk Standard Dilution Buffer 1	15 mL	2-8 °C
10X Penicillin Extraction Buffer	2 x 25 mL	2-8 °C
10X Balance Buffer	10 mL	2-8 °C

Sensitivity (Detection Limit)

Sample Type	Detection Limit (ng/g or ppb)
Meat/Fish Tissue	0.8
Milk	0.4
Milk Powder	0.4
Butter, Cheese, Curd	0.4
Yogurt, Buttermilk, Soured Milk, Whey, Sour Cream	0.4
Serum, Plasma	1.6
Swipe Sample for Surface Contamination	0.8
Urine	0.8

Specificity (Cross-Reactivity)

Analytes	Cross-Reactivity (%)
Penicillin G	100
Ampicillin	92
Azlocillin	112
Piperacillin	70
Nafcillin	55
Amoxicillin	44
Cloxacillin	40
Oxacillin	40
Cefoperazone	34
Ceftiofur	25

Required Materials Not Provided With the Kit

- Microtiter Plate Reader with 450 nm primary fi lter and optional 630 nm differential filter (MaxSignal® 4302 Plate Reader Catalog #FOOD-6003-01)
- Tissue Homogenizer
- Vortex Mixer
- 10, 20, 100 and 1000 µL Pipettes
- Distilled or deionized water
- Multi-Channel Pipette: 50–300 µL (Optional)
- Reagent Resevoir (Optional)
- Hexane, CAS 110-54-3 (ACS-grade or higher recommended)

Warnings and Precautions

PerkinElmer recommends reading the following warnings and precautions to ensure full awareness of ELISA techniques and other details while running the assay. More information can also be found in Troubleshooting section. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the procedures that come with this kit. If further assistance is required, please contact your local distributor or PerkinElmer at Bioo.Support@PerkinElmer.com

- The standards contain Penicillin G. Handle with particular care.
- Do not use the kit past the expiration date.
- Do not mix reagents from different kits or lots.
- Standards, antibodies, and plate are kit and lot specific.
- Ensure any antibodies and corresponding diluents are mixed in correct volumes.
- Maintain a laboratory temperature of 20 – 25 °C.
- Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation.
- Do not run assays in direct sunlight, as this may cause excessive heat and evaporation.
- Do not incubate the plate on a cold surface. Use several layers of paper towel or some other insulation material under the plates during incubation.
- Use only distilled or deionized water since water quality is very important.
- When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.
- Incubations should be timed as precisely as possible. Pipette with a consistent force and rate.
- Store unused plates at 2–8°C in sealed bags with a desiccant to maintain stability.
- Allow the plate to equilibrate to room temperature (20–25°C) in the sealed bag to prevent condensation.
- For small volume reagents (less than 2 mL), after mixing, briefly centrifuge to bring the solution to the bottom of the tube

SAMPLE PREPARATION

Be sure samples are properly stored. In general, samples should be refrigerated at 2–8°C. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples should be thawed at room temperature (20–25°C) or in a refrigerator before use. For information related to additional sample preparation methods, contact Bioo.Support@PerkinElmer.com.

1. **Preparation of 1X Penicillin Extraction Buffer:**

   Mix 1 volume of 10 X Penicillin Extraction Buffer with 9 volumes of distilled/deionized water.

2. **Preparation of 1X Balance Buffer:**
   Mix 1 volume of 10 X Balance Buffer with 9 volumes of distilled/deionized water.

When preparing solutions, mix vigorously for at least one minute in an air-tight container.

Meat/Fish Tissue

1. Homogenize a reasonable amount of sample with a suitable mixer until the sample has a consistency like paste. Store sample at -20°C until analyzed.
2. Weigh out 1 g of the homogenized sample and mix with 9 mL of 1X Penicillin G Extraction Buffer then vortex for 3 minutes or shake for 20 minutes with a shaker.
3. Centrifuge for 10 minutes at 4,000 x g at room temperature (20–25°C).
4. Take out 1 mL of supernatant and mix it with 2 mL of hexane, vortex for 2 minutes.
5. Centrifuge sample for 10 minutes at 4,000 x g.
6. Discard the top hexane layer and use 50 µL of the low aqueous layer per well in the test. Be sure to remove the entire upper, hexane layer by careful pipetting.

Note: *Dilution factor = 10*

Milk

For fat-free milk, dilute the milk sample 1:4 with 1X Penicillin Extraction Buffer (e.g. 100 µL of milk + 400 µL of 1X Penicillin Extraction Buffer). Use 50 µL of the diluted sample per well for the assay.

For regular milk with fat, centrifuge the milk sample at 4,000 x g for 10 minutes and discard the upper fat layer. A toothpick, pipette tip, or weighing spatula is generally adequate to drag the fat layer to the side or pierce an opening through which you may obtain the separated milk. Dilute the milk sample 1:4 with 1X Penicillin Extraction Buffer (e.g. 100 µL of milk + 400 µL of Penicillin Extraction Buffer). Use 50 µL of the diluted sample per well for the assay.

Note: *Dilution factor = 5*

Milk Powder

To 1 g of milk powder in a vial, add distilled water to 10 mL, dissolve sample completely by shaking. Then transfer 100 µL of dissolved milk sample above to a new tube, add 400 µL of Penicillin Extraction Buffer. Use 50 µL of the diluted sample per well for the assay.

Note: *Dilution factor = 5 (corresponding to the dissolved liquid milk)*

Butter/ Cheese/ Curd

1. To 1 g of finely grated cheese or butter, add 4 mL 1X Penicillin Extraction Buffer. (*For butter samples, it may be necessary to heat the sample in a warm water bath for several minutes to form a homogeneous solution before vortexing*).
2. Vortex vigorously for 5 minutes manually or using a multi-vortexer.
3. Centrifuge the samples for 10 minutes at 4,000 rpm.
4. Transfer 1 mL of the supernatant to a new tube.
5. Use 50 µL of the sample per well for the assay.

Note: *Dilution factor = 5*

Yogurt/ Buttermilk/ Soured Milk/ Whey/ Sour Cream

1. To 1 mL of the sample add 1 mL of 1X Balance Buffer and 3 mL of 1X Penicillin Extraction Buffer, vortex for 3 minutes at maximum speed.
2. Centrifuge for 5 minutes at 4,000 rpm.
3. Use 50 µL of the lower, aqueous layer for the assay (avoid contact with the top fat layer).

Note: *Dilution factor = 5*

Serum/Plasma

1. Centrifuge the serum/plasma sample at 4,000 rpm for 15 minutes.
2. Dilute the supernatant 1:19 with 1X Penicillin Extraction Buffer (e.g. 10 µL of serum/plasma + 190 µL of Buffer).
3. Use 50 µL of the diluted sample per well for the assay.

Note: *Dilution factor = 20*

Swipe Sample for Surface Contamination

1. Spray 1 mL of 1X Penicillin Extraction Buffer onto the contaminated surface, wipe the surface with one piece of Kimwipe paper thoroughly.
2. Put the Kimwipe paper to a 50-mL plastic tube and add 9 mL of 1X Penicillin Extraction Buffer.
3. Vortex for 3 minutes vigorously and leave it at room temperature for at least 15 minutes
4. Centrifuge the sample at 4,000 rpm for 15 minutes.
5. Use 50 µL of the supernatant per well for the assay.

Note: *Dilution factor = 10*

Urine

1. Centrifuge the urine sample at 4,000 rpm for 10 minutes.
2. Dilute the supernatant 1:9 with 1X Penicillin Extraction Buffer (e.g. 20 µL of urine + 180 µL of buffer)
3. Use 50 µL of the supernatant per well for the assay.

Note: *Dilution factor = 10*

PENICILLIN ELISA TEST KIT PROTOCOL

Reagent Preparation

IMPORTANT: All reagents and samples should be brought to room temperature at least 2 hours before use. Make sure to read the "Warnings & Precautions" section. Reagents and samples should be prepared prior to running ELISA. All reagents and samples should be mixed by inverting or vortexing prior to use. Prepare volumes that are needed for the number of wells being run. Do not return any reagents to the original bottles. Aliquotting reagents for individual use and using disposable reservoirs can minimize the risk of contamination and is recommended.

1. **Preparation of 1X Wash Solution**
   Combine 1 volume of the 20X Wash Solution with 19 volumes of distilled/deionized water. Mix well.

2. **Preparation of 1X HRP-Conjugated Antibody #2**
   Combine 1 volume of 200X HRP-Conjugated Antibody #2 with 199 volumes of Antibody #2 Diluent. Vortex for 10 seconds to mix. ***Prepare this solution immediately before performing its addition step in the ELISA.***

3. **Preparation of Penicillin G Standards**
   Add 1 mL of 1X Penicillin Extraction Buffer to the stock vial (1,000 ng) and mix by vortexing for 30 seconds to obtain 1,000 ppb stock. Centrifuge the tube briefly to re-collect the liquid. From the stock 1,000 ppb tube take 20 µL and dilute it into 980 µL of 1X Penicillin Extraction Buffer to make a 20 ppb stock in a glass vial (please do not use plastic vial). To make the working standards, serially dilute the 20 ppb standard stock vial in the provided empty standard vials as described in the following table. Make sure to mix each standard vial by vortexing thoroughly and briefly centrifuge before removing aliquots.
   For Preparing Standards for use with milk: Use the Milk Standard Dilution Buffer 1 for all dilutions after the 20 ppb stock, instead of 1X Penicillin Extraction Buffer.

Working Standards	Penicillin G Source	Volume of source to be added	Volume of Diluent
1.2 ppb vial	20 ppb stock vial	60 µL	940 µL
0.8 ppb vial	1.2 ppb vial	500 µL	250 µL
0.4 ppb vial	0.8 ppb vial	500 µL	500 µL
0.2 ppb vial	0.4 ppb vial	500 µL	500 µL
0.08 ppb vial	0.2 ppb vial	500 µL	750 µL
Negative Control vial	N/A	0 µL	500 µL

Note: The 1,000 ppb stock vial can be divided into single-use aliquots and stored at -20°C in a non-frost-free freezer for at least 1 month. The working standards must be freshly prepared before use in the ELISA. After using the standards, empty the standard vials and store them at 4°C. The empty vials can be re-used for preparing standards for subsequent assays.

4. **Preparation of 1X Penicillin Capture Protein**

   Mix 1 volume of 10X Penicillin Capture Protein with 9 volumes of Penicillin Capture Protein Diluent. Prepare 5 minutes before use.

ELISA Testing Protocol

1. Add 50 µL of each Penicillin G standard in duplicate to different wells using a new pipette tip for each standard addition. Add standards from low to high concentration.
2. Add 50 µL of each sample in duplicate to remaining wells using a new pipette tips for each sample addition.
3. Add 100 µL of 1X Penicillin Capture Protein to each well.
4. Mix the solution in the wells for 1 minute by using a plate shaker or by tapping the plate gently, as to not allow any liquid to spill over.
5. Cover the plate. Incubate the plate for 30 minutes at controlled room temperature (20–25°C).
6. After incubation, wash the plate 3 times.
   To wash the plate:
   a. Decant the liquid thoroughly from each well.
   b. Add 250 µL of 1X Wash Solution to each well.
   c. Incubate the wash solution for 15 seconds in each well.
   d. Decant the wash solution thoroughly from each well.
   e. Repeat Steps b.–d. two more times.
7. After the 3rd wash, invert the plate and forcibly tap it against paper towels until no 1X Wash Solution remains. Continue to the next step immediately to avoid drying of the plate.
8. Add 150 µL of freshly prepared 1X Antibody #2 solution to each well.
9. Mix the solution in the wells for 1 minute by using a plate shaker or by tapping the plate gently, as to not allow any liquid to spill over.
10. Cover the plate. Incubate the plate for 30 minutes at controlled room temperature (20–25°C).
11. After incubation, wash the plate 3 times.
    To wash the plate:
    a. Decant the liquid thoroughly from each well.
    b. Add 250 µL of 1X Wash Solution to each well.
    c. Incubate the wash solution for 15 seconds in each well.
    d. Decant the wash solution thoroughly from each well.
    e. Repeat Steps b.–d. two more times.
12. After the 3rd wash, invert the plate and forcibly tap it against paper towels until no 1X Wash Solution remains. Continue to the next step immediately to avoid drying of the plate.
13. Add 100 µL of TMB Substrate to each well. ***Avoid contamination by not touching the interior of the wells with the pipette tip. Discard the substrate if any coloration is observed before addition to each well***.
14. Mix the solution in the wells for 1 minute by tapping the plate gently, as to not allow any liquid to spill over.
15. Cover the plate. Incubate the plate for 15 minutes at controlled room temperature (20–25°C) in the dark.
16. After incubation, add 100 µL of Stop Solution to each well to stop the substrate reaction. ***Avoid contamination by not touching the interior of the wells with the pipette tip.***
17. Use a lint-free wipe to clean the bottom of the wells before continuing to the next step.
18. Obtain the absorbance values using a plate reader set at 450 nm primary filter (OD450). ***To decrease background with some readings, it is recommended to use an additional 630 nm differential filter simultaneously with each reading.***

Penicillin Concentration Calculations

A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.

$$\text{Relative absorbance (\%)} = \frac{\text{absorbance standard (or sample)} \times 100}{\text{absorbance zero standard}}$$

Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ng/mL from the standard curve. A special program with Excel functionality, MaxSignal® ELISA Analysis Program in Excel, is available upon request to evaluate the MaxSignal® ELISA test results. Please contact your local distributor or Bioo.Support@PerkinElmer.com for further information

TROUBLESHOOTING

No Color Development or No Signals with Standards

Possible Causes	Recommended Action
Wrong antibodies were used, or antibody #2 was prepared incorrectly or has deteriorated.	Make sure that the antibodies used are the ones that came with the kit. All antibodies are kit- and lot-specific. Make sure that the antibody #2 and diluent are mixed in correct volumes.

Low Optical Density (OD) Readings

Possible Causes	Recommended Action
Reagents were expired or mixed with a different lot number.	Verify the expiration dates and lot numbers.
Wash solution was prepared incorrectly.	Use the wash solution for the kit and make sure that it is prepared correctly.
Incubation times were too short.	Time each plate separately to ensure accurate incubation times, follow protocol.
Lab temperature was too low.	Maintain the lab room temperature within 20°–25°C (68°–77°F). Do not run assays under air conditioning vents or near cold windows.
Reagents and plates were too cold.	Make sure plates and reagents are brought up to room temperature. Keep the kit components out of the kit box for at least 2 hour before starting the assay.
Reader was at wrong wavelength, or reader was malfunctioning.	Make sure the wavelength is 450 nm for the assay and read the plate again. Verify reader calibration and lamp alignment.
Excessive kit stress has occurred.	Check records to see how many times the kit has cycled from the refrigerator. Check to see if the kit was left at extreme temperatures for too long.

High Background or High Optical Density (OD) Readings

Possible Causes	Recommended Action
Substrate solution has deteriorated.	Make sure the substrate is colorless prior to addition to the plate.
There was insufficient washing or poor washer performance.	Use the number of washes per the instructions. Ensure at least 250 µL of wash solution is dispensed per well for each wash. Verify the correct performance of any automatic washer system; repair as needed.
Reader was malfunctioning or not calibrated	Verify the reader's performance using a calibration plate and check the lamp alignment.
Lab temperature was too high.	Maintain the room temperature within 20 – 25 °C (68 – 77 °F). Avoid running assays near heat sources or in direct sunlight.

One or More of the Standard Curve Points Are Out of Range

Possible Causes	Recommended Action
Standards were added in wrong order or recorded in wrong position.	Follow the protocol and re-run the assay. Add standards to plate only in the order from low concentration to high concentration.
Standards were intermixed with other standards.	Ensure all standards used are from the same lot..

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