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Ampicillin ELISA Kit

rev *12/19*

(Catalog # E4350-100; 100 assays, Store kit at -20°C)

I. Introduction:

Ampicillin belongs to the beta-lactam antibiotic family and it irreversibly inhibits transpeptidase to prevent cell wall synthesis in bacteria. Ampicillin has broad spectrum activity against most gram-positive and gram-negative bacteria and is often used for treating bacterial infections such as respiratory tract infections, urinary tract infections and meningitis. Nevertheless, it has some common side effects including rash, nausea, diarrhea, seizures and anaphylaxis. The traditional techniques/instruments (HPLC or GC-MS) for detecting beta-lactam antibiotics are expensive, laborious, and time-consuming. Immunoassay techniques, such as ELISA, are commonly preferred as simple, reliable and rapid methods. BioVision's Ampicillin ELISA Kit is a competitive-based ELISA that can detect ampicillin in milk, urine, tissues and serum. It can detect and quantify broad range of ampicillin (5 – 625 ng/ml) within 90 minutes.

II. Applications:

In vitro, quantitative determination of beta-lactam antibiotics such as ampicillin
Detection Range: 5 - 625 ppb (ng/ml)
Sensitivity: 2.5 ppb
Detection limit: 10 ppb
Cross Reactivity: Penicillin G – 22%, Clavulanic acid – 0%, Pefloxacin – 0%, Kanamycin – 0%

III. Sample Type:

Serum, urine, milk and tissues (e.g. pork, liver, chicken, fish and shrimp)

IV. Kit Contents:

Components	E4350-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4350-100-1
Ampicillin Standard	2 vials	Yellow	E4350-100-2
HRP Conjugate Stock	25 µl	Blue	E4350-100-3
Antibody	7 ml	NM/Red	E4350-100-4
TMB substrate	12 ml	Amber	E4350-100-5
Stop Solution	10 ml	NM/Blue	E4350-100-6
Sample Diluent	20 ml	NM	E4350-100-7
Wash Buffer (10X)	50 ml	NM	E4350-100-8
Extraction Solution	2 ml	Brown	E4350-100-9
Standard Buffer	40 ml	WM	E4350-100-10
Conjugate Buffer	7.5 ml	NM/Green	E4350-100-11
Plate Sealers	4	--	E4350-100-12

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards and sample dilutions

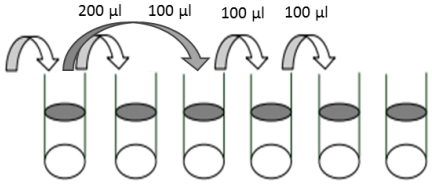
VI. Storage and Handling:

The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Opened kit is stable for 2 months at 4°C.

VII. Reagent and Standard Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Antibody, TMB Substrate, Stop Solution, Sample Diluent, Extraction Solution, Standard Buffer and Conjugate Buffer:** Ready to be used. After use, store them at 4°C.
- **HRP Conjugate Stock**: Spin briefly before opening the tube. Pipet 3 µl of HRP Conjugate Stock into Conjugate Buffer (7.5 ml) bottle to prepare conjugate working solution. Vortex the bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.
- **Wash Buffer (10X)**: Bring bottle to room temperature. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stored at 4°C for one month.
- **Ampicillin Standards**: Reconstitute the lyophilized Ampicillin standard by adding 1.2 ml of Standard Buffer to make the 625 ppb standard stock solution (S5). (Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely.) Prepare 0.4 ml of 312 ppb standard (S4) by adding 200 µl of the above stock in 200 µl Standard Buffer. Perform 5-fold serial dilutions from S5 (e.g. add 100 µl standards in 400 µl buffer) to prepare S3 to S1 standards sequentially. S0 is the Standard Buffer only. These standards are stable at -20°C for up to 3 weeks..



Tube #	S5	S4	S3	S2	S1	S0
Standard Buffer (µl)	1200	200	400	400	400	500
Conc. (ppb)	625	312	125	25	5	0

VIII. Sample Preparation:

- **Serum**

1. Add 20 µl of Extraction Solution to 200 µl of serum and vortex well.

2. Centrifuge the serum sample at 10,000 x g for 20 min at 4°C and recover the supernatant.
3. Dilute the supernatant 10 fold with Sample Diluent. For example, mix 20 µl of supernatant with 180 µl of Sample Diluent. Use 50 µl per well for the assay. (Note: Dilution factor: 10)

- **Urine**

1. Centrifuge 0.5 ml of urine at 10,000 x g for 5 min and recover the supernatant.
2. Dilute the supernatant 10 fold with Sample Diluent. For example, mix 20 µl of urine with 180 µl of Sample Diluent. Use 50 µl per well for the assay. (Note: Dilution factor: 10)

- **Milk**

1. Add 20 µl of Extraction Solution to 1 ml of milk and vortex well.
2. Centrifuge the sample at 10,000 x g for 20 min at 4°C and recover the clear supernatant.
3. Dilute the supernatant 10 fold with Sample Diluent. For example, mix 20 µl of the supernatant with 180 µl of Sample Diluent. Use 50 µl per well for the assay. (Note: Dilution factor: 10)

- **Tissue (pork, liver, chicken, fish and shrimp)**

1. Weigh 1 g of the tissue sample. Mix the tissue with 1 ml of water and 20 µl of Extraction Solution. Homogenize and vortex for 5 min.
2. Centrifuge the sample at 10,000 x g for 20 min at 4°C and recover the supernatant.
3. Dilute the supernatant 10 fold with Sample Diluent. For example, mix 20 µl of the supernatant with 180 µl of Sample Diluent Use 50 µl per well for the assay. (Note: Dilution factor: 10)

IX. Ampicillin ELISA Assay Protocol:

Notes: It is recommended that all standards and samples should be run in duplicate. A Standard curve must be run each time an assay is performed.

1. Prepare all reagents, standards and samples as sections VII and VIII respectively.
2. Add 50 µl of Standards or Samples per well. Then add 50 µl of conjugate working solution and 50 µl of Antibody to the above wells.
3. Cover the microtiter plate with plate sealer and mix well. Incubate the plate at room temperature (25°C) for 60 min.
4. Aspirate all reagents and wash each well 5 times: add 250 µl of 1X Wash Buffer and incubate for 30 seconds. Remove 1X Wash buffer completely before the next wash. (Complete removal of wash buffer is essential for accurate results.) Repeat this step 4 more times.
5. Add 100 µl of TMB Substrate to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no ampicillin (S0). When its reading is approximately between 0.8 and 1.0 (usually between 5-30 min after adding the TMB Substrate), add 50 µl of Stop Solution and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm.

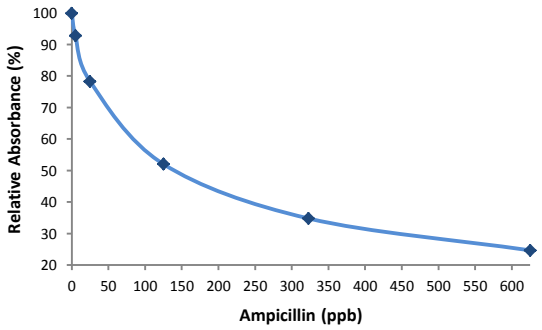
X. Calculation:

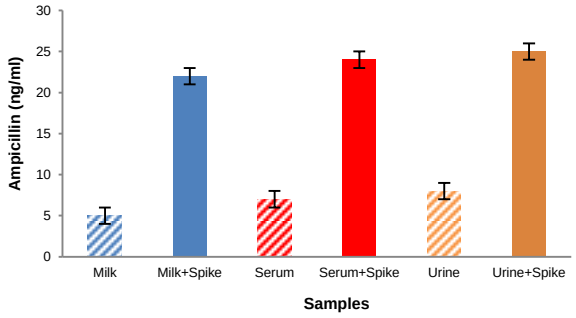
The mean values of relative absorbance are divided by the absorbance value of the zero-standard (A_0) and multiplied by 100%. The zero-standard is set to 100% and the relative absorbance of the standards and samples (A) are expressed as percentages.

Relative Absorbance (%) = A/A_0 X 100%
A: The average absorbance of the standards or samples
A_0: The average absorbance of the zero standard

The Standard Curve is done by plotting the relative absorbance of the standards vs. ampicillin concentrations. The concentration of ampicillin of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor.

A.





Figures. **A.** Ampicillin standard curve (*This standard curve is for demonstration only. A standard curve must be run with each assay*). **B.** Spike recovery experiment: Milk, human serum and urine samples were assayed with and without spike (20 ng/ml) and showed 80-100% recovery.

XI. RELATED PRODUCTS:

Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
Folic Acid ELISA Kit (Cat. No. E4523-100)
Caffeine Acid ELISA Kit (Cat. No. E4558-100)
Penicillin G sodium (Cat. No. 2503-100, 500)
Quinolone ELISA Kit (Cat. No. E4530-100)
Vancomycin ELISA Kit (Cat. No. E4605-100)

FOR RESEARCH USE ONLY! Not to be used on humans.