Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Amoxicillin ELISA Kit

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(Catalog # E4614-100, 100 assays, Store at 4°C)

I. Introduction:

Amoxicillin is an antibiotic useful for the treatment of a number of bacterial infections. It is the first line treatment for middle ear infections. It may also be used for strep throat, pneumonia, skin infections, and urinary tract infections. Common side effects include nausea, rash and increase the risk of yeast infections. Among other standard techniques/instruments (HPLC or GC-MS) are utilized to detect Amoxicillin. However, these techniques are complex, expensive, laborious, and time-consuming. Immunoassay techniques, such as ELISAs are commonly preferred as a simple, reliable and rapid method for the quantification of Amoxicillin in various samples. BioVision's Amoxicillin ELISA Kit is a competitive-based ELISA that can be used for the determination of this antibiotic in tissue, egg.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Amoxicillin
Detection Range: 1 - 27 ppb
Sensitivity: 1 ppb
Detection limitation: 20 ppb for tissue, 10 ppb for egg

III. Sample Type:

Tissue, egg

IV. Kit Contents:

Components	E4614-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4614-100-1
Standard (S0 – S4)	1 ml X 5	E4614-100-2
Enzyme Conjugate (11X)	0.7 ml	E4614-100-3
Enzyme conjugate dilution	7 ml	E4614-100-4
Substrate A	6 ml	E4614-100-5
Substrate B	6 ml	E4614-100-6
Stop Solution	6 ml	E4614-100-7
Wash Buffer (20X)	30 ml	E4614-100-8
Redissolving Solution	30 ml	E4614-100-9

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, HCl, N,N-Dimethylformamide (DMF)
- Microplate reader capable of measuring absorbance at 450 nm
- Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer:** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 ml of Wash Buffer (20X) into 570 ml deionized or distilled water to prepare 600 ml of Wash Buffer (1X). Keep it at 4°C for one month.
2. **Enzyme Conjugate:** take 1 part 11X Concentrated Enzyme conjugate, add 10 parts Enzyme conjugate dilution, dilute at 1:10.
3. **Standards Concentration:** Ready to use

Standards	S0	S1	S2	S3	S4
Concentration (ppb)	0	1	3	9	27

4. **Sample Preparation:**
Note: The prepared sample maybe stored for up to one day at 2-8°C.

A. Tissue (chicken)

Take 1g tissue sample into 10ml Polystyrene centrifuge tube; add 4ml deionized water,vortex in high-speed for 1min;centrifuge at above 3000g at room temperature for 10min. Take 200 ul up-layer clear liquid into 2ml Polystyrene centrifuge tube, add 200 ul redissolving solution, vortex for 10s and mix evenly. Take 50 µl up-layer liquid for analysis. (Dilution factor: 10)

B. Egg

Take 1 g egg sample into 10ml Polystyrene centrifuge tube; add 3 ml 40% Methanol Solution, vortex in high-speed for 2 min. Centrifuge at above 3000xg at room temperature for 10min. Take 200 ul up-layer clear liquid into 2 ml Polystyrene centrifuge tube, add 200 ul redissolving solution, vortex for 10s and mix evenly. Take 50 µl for further analysis. (Dilution factor: 8)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 50 µl of the sample and standard solution to separate duplicate wells; Then 50 µl enzyme conjugate into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25°C for 30 min.
3. Wash the microplate with the washing buffer at 250 µl/well for 4-5 times. Each time soak the well with the washing buffer for 15-30 sec, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
4. Add 100 µl mixture of the substrate A and substrate B into each well (Note: mix Substrate A, and Substrate B at 1:1, the mixture should be used in 10min, never use metal container or metal to stir the solution, otherwise the substrate may be invalid.). Mix gently by shaking the plate manually, and incubate at 25°C for 15 minutes at dark for coloration.
5. Add 50 µl of the stop solution into each well. Mix gently by shaking the plate manually. Read the OD value at the dual-wavelength 450/630nm within 5 min.

IX. Calculation:

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

Absorbance Value (%) = B/B_0 X 100%

B: The average absorbance value of the sample or standard

B_0: The average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Fluoroquinolones standards solution (ppb) as x-axis. The Fluoroquinolones concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

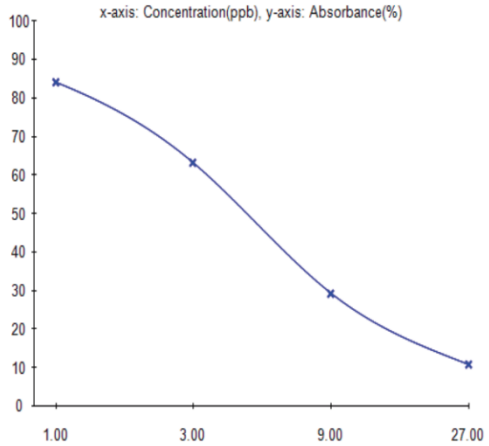

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. Related Products:

- Ampicillin ELISA Kit (Cat. No. E4350-100)
- Streptomycin ELISA Kit (Cat. No. E4272-100)
- Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
- Kanamycin ELISA Kit (Cat. No. K4210-100)
- Quinolone ELISA Kit (Cat. No. E4530-100)