Amoxicillin ELISA Kit

Product #: E6202, 96T

For research use, for professional use. Not for IVD or therapeutic purpose.

Manufacturer Information

Ring Biotechnology Co., Ltd

E-mail: export@ringbio.com diego@nbgen.com Web: www.ringbio.com

Add: Building 3, Zhongtongtai TechnoPark, No. 11, Kechuang 14th St, Beijing 100176, CHINA

Tel: +86-10-56267496 Technical Support & Service: +86-18600362934

1. Description

Amoxicillin is a broad-spectrum antibiotic that is used for treating bacterial infections. However, due to the severe allergic reactions, it is being restricted and replaced by other antibiotics. Now in all major countries, strict maximum residue limits have been set on amoxicillin.

This current amoxicillin ELISA Kit is based on indirect competitive ELISA to detect amoxicillin residue in cell culture, milk and honey. In the kit, amoxicillin antigen is coated on the microtiter well and samples contained the drug will compete for amoxicillin specific antibody with the antigen. After washing and adding the enzyme conjugate, TMB substrate is used to show the reaction color. The concentration of amoxicillin in sample is inversely proportional to the OD value, which can be interpreted with a standard curve.

2. Application

This kit is applicable for determination of amoxicillin in raw milk, milk powder, honey and tissue(pork, chicken, beef, mutton). Other applications can also be developed upon request.

3. Kit components

1) Microtiter plate, 96wells, 1 plate
2) Amoxicillin standards, 1mL, 0.00 ng/mL
3) Enzyme conjugate, 7mL, with brown cap
4) TMB Substrate, 12mL, with brown cap
5) Stop solution, 7 mL
6) 20x Wash buffer, 50mL
7) 2x tissue sample buffer, 50mL
8) 20x honey sample buffer, 50mL

4. Instrument and material required

1) ELISA reader, with 450/630nm
2) Centrifuge
3) Balance, 0.0001g
4) Centrifuge tube, 2mL, 15mL
5) Vortex mixer
6) Micropipette, 5-50μL, 20-200μL, 100-1000μL
7) Multi-channel pipette, 300μL
8) graduated pipette,10mL

5. Reagent required

Deionized water, absolute methanol, concentrated hydrochloric acid

6. Buffer preparation

Buffer 1: Wash buffer:

Dilute 20x wash buffer with deionized water, in the volume ratio of 1:19, for example, 10mL 20x wash buffer + 190mL deionized water, mix thoroughly.

This diluted wash buffer can be stored at 4°C for 1 month.

Buffer 2: 1x tissue sample buffer

Dilute 2x sample buffer with deionized water, in the volume ratio of 1:1, for example, 10mL 2x sample buffer + 10mL deionized water, mix thoroughly.

This tissue sample buffer can be stored at 4°C for 1 month.

Buffer 3: 1x honey sample buffer

Dilute 20x sample buffer with deionized water, in the volume ratio of 1:19, for example, 10mL 20x sample buffer + 190mL deionized water, mix thoroughly and add 10% absolute methanol.

This honey sample buffer can be stored at 4°C for 1 month.

0.1M hydrochloric acid: add 0.83mL concentrated hydrochloric acid into 100ml deionized water, mix well.

7. Sample preparation

7.1 Precautions before prepare samples:

1) Use disposable tips during the test. Change new tip for different sample / reagent.
2) Make sure all lab wares are clean and ready to use.
3) Prepared sample shall be analyzed immediately after dilution.

7.2 Milk

Take 1mL raw milk into 2mL centrifuge tube, add 60μL 0.1M hydrochloric acid, mix well, take 50μL for assay.

7.3 Milk powder

Take 1.0±0.05g sample into a new 15mL centrifuge tube, add 8mL deionized water, vortex for 3min to dissolve, take 1mL milk into 2mL centrifuge tube, add 50μL 0.1M hydrochloric acid, mix well with vortex mixer, take 50μL for assay.

7.4 Honey

Take 1.0±0.05g honey sample into a new 15mL centrifuge tube, add 10mL 1x honey sample buffer (**buffer 3**), mix thoroughly by vortex for 2min. Take **500μL** supernate and further dilute with **500μL** 1x honey sample buffer (**buffer 3**), vortex for 10s, and then take 50μL for assay.

7.5 Tissue(pork, chicken, beef, mutton)

Weight 1±0.05g sample into a 15mL centrifuge tube, add 2ml absolute methanol, vortex for 2min; then centrifuge at 5000rpm for 5min, take 100μL supernate into 2mL centrifuge tube, add 900μL 1x tissue sample buffer, vortex for 10s, mix fully. Take 50μL for assay.

8. Notice and precautions before assay

1) Make sure the ELISA kit and all reagents are returned to room temperature (20-25 °C). For example, keep these reagent and kits at room temperature for at least 60min.
2) Return unused kit components to 2-8 °C.
3) Washing step is important for the reproducibility of the kit, please follow this instruction carefully.
4) Cover the ELISA plate during all incubation. Avoid direct sunlight.

9. Assay procedures

1) Return the ELISA kit and all reagents to room temperature (20-25 °C). For example, keep these reagent and kits at room temperature for at least 60min.
2) The sample buffer, wash buffer shall also be brought to room temperature.
3) Take needed microwells and zip rest in the zip-bag and return to 2-8 °C.
4) Layout the plate and record sample and standard well positions. It is recommended to run all tests in duplicates.
5) **Add sample/standard/enzyme conjugate**: add sample/standard into the wells, 50μL per each, then add enzyme conjugate solution, 50μL per well, shake gently and then cover the plate and incubate at **37 °C for 45min**.
6) **Wash**: take out the plate and pour the liquid out. Use the diluted wash buffer (**buffer 1**) to wash the plate, 250μL/well. Wash for 4-5 times with interval of 10s. The pour the liquid out and tap the plate against absorbent paper. Eliminate the air bubble in the wells with micropipette tip if the bubble exists.
7) **Coloration**: add TMB substrate, 100μL per well, and then cover the plate and incubate **25 °C**

for 15min.

8) **Stop the reaction**: add stop solution, 50μL per well, shake gently and read the plate with ELISA reader at **450nm**. Read the plate within 5min after adding stop solution.

10. Result Calculation

10.1 Standard curve calculation

Based on the OD of the standard (0.00ng/mL) and measure the OD of the remaining standards.

OD(0.5ng/mL)=OD(0.00ng/mL)x0.68

OD(1.5ng/mL)=OD(0.00ng/mL)x0.48

OD(4.5 ng/mL)=OD(0.00ng/mL)x0.25

OD(13.5 ng/mL)=OD(0.00ng/mL)x0.15

OD(40.5 ng/mL)=OD(0.00ng/mL)x0.08

10.2 Qualitative estimation

This kit is based on competitive ELISA, thus the OD values is inversely proportional to the amoxicillin content contained in sample. If there is no ELISA reader, just compare the color of sample with the amoxicillin Standard to get the estimated sample amoxicillin content.

10.3 Quantitative calculation

With ELISA reader, a standard curve can be plotted with the ODs obtained. Use Logit-log, Cubic spline or logistic curve, etc to calculate the amoxicillin sample content.

Usually, this software will be installed with your ELISA reader. If it is not provided, please contact us for help, spreadsheet with Logit-log calculation will be provided upon your request.

11. Sample dilution factor:

Milk: 1

Milk powder: 9

Honey: 20

Tissue: 30

12. Specifications of the kit

1) **Sensitivity**: 0.5ng/mL
2) **Specificity**:
   Amoxicillin: 100%;
   Benzathine penicillin: 325%
   Penicillin G: 610%,
   Ampicillin: 189%;
   Carbenicillin: 28%
   Cefazolin: 15%
   Cefradine: <0.1%
   Cefotaxime: 9%
3) **Limit of Detection**:
   Milk: 0.5 ng/mL
   Milk powder: 4.5ng/mL
   Honey: 10ng/mL
   Tissue: 15ng/mL
4) **Recovery**: 70%-130%
5) **Precision**: C.V<10%.

13. Cautions and tips for the test

1) Lower room temperature, e.g., lower than 20 °C may cause lower OD values. Please make sure all reagent and kit components are returned to room temperature.
2) Wash step is vital for the reproducibility of the kit. Please wash according to the kit instruction. Do not let the plate dry during wash. Continue the next operations immediately after wash step.
3) Shake each reagent gently before use.
4) Stop solution is acidic, please handle with care.
5) Do not use expired kits and reagents. Do not mix the reagent and kits from different LOT.
6) The kit is stored at 2-8°C(36-46°F), do not freeze.
7) TMB substrate is sensitive to sunlight. Avoid direct sunlight.
8) If Standard 1 (0ng/L) OD is lower than 0.5, do not use. The kit may be expired or deteriorated.

9) The coloration step takes 15min. You can prolong it to 20min-25min if the color of the well is too light. On the contrary, please reduce the incubation time.
10) The incubation is 37 °C, lower or higher temperature will cause changes of OD and sensitivity of the kits, which may affect the result of the assay.

14. Storage and expiration

The kit is valid for 12months when stored at 2-8 °C. LOT and Expiry information are printed on the package.