Competitive Enzyme Immunoassay Kit for Quantitative Analysis of Amoxicillin

1. Background

Amoxicillin, also spelled amoxycillin, 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 among others. It is taken by mouth, or less commonly by injection.

Common side effects include nausea and rash. It may also increase the risk of yeast infections and, when used in combination with clavulanic acid, diarrhea. It should not be used in those who are allergic to penicillin. While usable in those with kidney problems, the dose may need to be decreased. Its use in pregnancy and breastfeeding does not appear to be harmful.

2. Test Principle

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Amoxicillin residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the Amoxicillin in it, after comparing with the Standard Curve, multiplied by the dilution factor, Amoxicillin quantity in the sample can be calculated.

3. Applications

This kit can be used in quantitative and qualitative analysis of Amoxicillin in animal tissue (pork, chicken) , milk and egg.

4. Cross-reactions

Amoxicillin……………....……….……………….........100%

5. Materials Required

5.1 Equipments

----Microtiter plate spectrophotometer (450nm/630nm)
----Rotary evaporator or nitrogen gas drying system
----Homogenizer
----Shaker
----Vortex mixer
----Centrifuge
----Analytical balance (inductance: 0.01g)
----Graduated pipette: 10ml
----Rubber pipette bulb
----Polystyrene centrifuge tubes: 2ml, 50ml
----Glass test tube: 10ml
----Volumetric flask: 100ml, 500ml
----Micropipettes: 20ul-200ul,100ul-1000ul, 250ul-multipipette

5.2 Reagents

---- Disodium hydrogen phosphate 12-hydrate ($Na_2HPO_4 \cdot 12H_2O$, AR)
---- Sodium dihydrogen phosphate dehydrate (NaH_2PO_4, AR)
---- Methanol(AR)
----Deionized water

6. Kit Components

- Microtiter plate with 96 wells coated with antigen
- Ceftiofur standard solutions. (1ml×6 bottles)

0 ppb, 1.5ppb, 4.5ppb, 13.5ppb, 40.5ppb, 121.5 ppb

- Spiking standard solution: 1ml, **1ppm**
- Enzyme conjugate (12ml)……..…..……….....red cap
- Antibody solution (7ml)……………….……green cap
- Solution A (7ml) ……………………………..white cap
- Solution B (7ml) …………………………..…red cap
- Stop solution (7ml) …………………………yellow cap
- 20×Concentrated wash solution (40ml) ………………………………………… transparent cap
- 2×Concentrated extraction solution (50ml) ………………………………………………. blue cap

7. Reagents Preparation

Solution 1: pH=7.2 0.02 M PBS

Weigh 2.58g disodium hydrogen phosphate 12-hydrate, 0.435g sodium dihydrogen phosphate dihydrate, dilute with deionized water to 500ml.

Solution 2: 0.03 M PBS

Weigh 3.87g disodium hydrogen phosphate 12-hydrate, 0.654g sodium dihydrogen phosphate dihydrate, dilute with deionized water to 500ml.

Solution 3: Tissue extraction solution

Mix 10ml of methanol and 70ml of 0.03M

Kwinbon Biotech

PBS(solution 2) completely.

Solution 4: Raw Milk extraction solution

Mix 8ml of methanol and 2ml of 0.02M PBS(solution 1) completely.

Solution 5: UHT Milk extraction solution

Mix 3ml of methanol and 7ml of 0.02M PBS(solution 1) completely.

Solution 6: Extraction solution

Dilute the 2×concentrated extraction solution with deionized water in the volume ratio of 1:1(*e.g. 10ml of 2×extraction solution + 10ml of deionized water*), which will be used for sample extraction, *this solution can be stored at 4℃for 1 month*.

Solution 7: Wash solution

Dilute the 20×concentrated wash solution with deionized water in the volume ratio of 1:19(*e.g. 5ml of 20×wash solution + 95ml of deionized water*), which will be used for washing the plates. *This solution can be stored at 4℃for 1 month*.

8. Sample Preparations

8.1 Notice and precautions before operation

(a) Please use one-off tips in the process of experiment, and change the tips when absorbing different reagent.

(b) Make sure that all experimental instruments are clean.

8.2 Tissue (pork, chicken)

----Take 2.0±0.05g of homogenized tissue sample into a 50ml tube, then add 8ml of tissue extraction solution (**solution** 3), shake for 5min, and then centrifuge for separation: 3000g / ambient temperature / 5min..

----Transfer 500μl of the supernate into a 2ml polystyrene centrifuge tube, add 500μl of extraction solution(solution 6), vortex for 30s to mix completely.

----Take 50μl per well for assay.

Dilution factor: 10

8.3 Milk

8.3.1 Raw milk

----Take *1ml* of milk into a *2ml* polystyrene centrifuge tube, add *1ml* of raw milk extraction solution(**solution 4**), vortex for *30s* to mix completely.

----Transfer 500*μl* of the solution into a 2ml polystyrene centrifuge tube, add 500*μl* of extraction solution(extraction 6), vortex for 30s to mix completely.

----Take 50μl per well for assay.

8.3.2 UHT milk

----Take *1ml* of UHT milk into a *2ml* polystyrene centrifuge tube, add *1ml* of UHT milk extraction solution(**solution 5**), vortex for *30s* to mix completely.

----Transfer 500*μl* of the solution into a 2ml polystyrene centrifuge tube, add 500*μl* of extraction solution(**solution 6**), vortex for 30s to mix completely.

----Take 50μl per well for assay.

Dilution factor: 4

8.4 Egg

----Homogenize the egg white and yolk with homogenizer.

----Weigh 1.0±0.05g of the homogenate into a 10ml polystyrene centrifuge tube, add 2ml of deionized water, vortex for 1min, then centrifuge: 5min / 3000g / 20-25℃.

----Transfer 200μl of the supernate into a 2ml polystyrene centrifuge tube, add 200μl of extraction solution(**solution 6**), vortex for 1min to mix completely(*The prepared solution should be white or light yellow*).

----Take 50μl per well for assay.

Dilution factor: 6

9. Assay process

9.1 Notice before assay

9.1.1 Make sure all reagents and microwells are all at room temperature (20-25℃).

9.1.2 Return all the rest reagents to 2-8℃ immediately after used.

9.1.3 Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.

9.1.4 Avoid the light and cover the microwells during incubation.

9.2 Assay Steps

9.2.1 Take all reagents out at room temperature (20-25℃) for more than 30min, homogenize before use.

9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.

9.2.3 The diluted wash solution should be rewarmed to be at room temperature before use.

9.2.4 **Number:** Numbered every microwell positions and all standards and samples should be run in duplicate.

Kwinbon Biotech

Record the standards and samples positions.

9.2.5 **Add standard solution/sample and antibody solution**: Add 50μl of standard solution(**Kit provided**) or prepared sample to corresponding wells. Add 50μl of antibody solution(**Kit provided**). Mix gently by rocking the plate manually and incubate for 30min at 25℃ with cover.

9.2.6 **Wash**: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250μl diluted wash solution (**solution** 7) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (*the rest air bubble can be eliminated with unused tip*).

9.2.7 **Add Enzyme conjugate:** Add 100μl of enzyme conjugate(**Kit provided**) to each well, Mix gently by rocking the plate manually and incubate for 30min at 25℃ with cover. *Repeat the wash step again*.

9.2.8 **Coloration**: Add 50μl solution A(**Kit provided**) and 50μl solution B(**Kit provided**) to each well. Mix gently by rocking the plate manually and incubate for 15min at 25℃ with cover(see 12.8).

9.2.9 **Measure**: Add 50μl of stop solution(**Kit provided**) to each well. Mix gently by rocking the plate manually and measure the absorbance at 450nm (*It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution*).

10. Results

10.1 Percentage absorbance

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.

$$\text{Absorbance}(\%) = \frac{B}{B_0} \times 100\%$$

B ——absorbance standard (or sample)

B0 ——absorbance zero standard

10.2 Standard Curve

----To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the amoxicillin standards solution (ppb) as x-axis.

----The amoxicillin 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.

Please notice:

For evaluation of the result, special software has been developed, which can be provided on request.

11. Sensitivity, accuracy and precision

Test Sensitivity: **1.5ppb**

Detection limit

Animal tissue……………………………………………15ppb

Milk……………………………………………………6ppb

Egg……………………………………………………9ppb

Accuracy

Tissue(chicken)……………………………………100±20%

Tissue(pork)…………………………………………90±20%

Milk(raw Milk)……………………………………… 100±20%

Milk(UHT Milk)………………………………………90±20%

Egg……………………………………………………100±20%

Precision

Variation coefficient of the ELISA kit is less than 10%.

12. Notice

12.1 The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25℃).

12.2 Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.

12.3. Shake each reagent gently before use.

12.4. Keep your skin away from the stop solution for it is the 0.5M H_2SO_4 solution.

12.5 Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

12.6 Keep the ELISA kits at 2-8℃,do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

12.7 Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5(A450nm<0.5).

12.8 The coloration reaction needs 15min after the addition of solution A and solution B. And you can prolong the incubation time from 20min to more if the color is too

Kwinbon Biotech

light to be determined. Never exceed 25min, on the contrary, shorten the incubation time properly.

13. Storage

Storage condition: 2-8℃.

Storage period: 12 months

Kwinbon Biotech