VERSION 1.01

AMPICILLIN ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Amplicillin ELISA Test Kit

Catalogue Number. IP100017

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Ampicillin in samples. The antigens conjugated Ampicillin is pre-coated on the micro-well stripes, Ampicillin in the sample and the conjugated antigens pre-coated on the micro-well stripes compete for the anti-Ampicillin antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with Ampicillin concentration in the sample. This value is compared to the standard curve and concentration of Ampicillin in the sample is subsequently obtained.

Technical specifications

Sensitivity: 0.1 ppb
Incubator temperature: 25℃
Incubator time: 30min~30min~15min
Detection limit: Tissue 2 ppb
Cross-reaction rate: Ampicillin 100%, Benzyl penicillin 0.8%, Cloxacillin 0.2%, Dicloxacillin 0.1%
Amoxicillin 0.1%, Ceftiofu 0.1%
Recovery rate: Tissue 60-120%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb and 8.1ppb	
3	Enzyme conjugate	7ml	red cap
4	Antibody working solution	7ml	blue cap
5	Substrate A	7ml	white cap
6	Substrate B	7ml	black cap
7	Stop solution	7ml	yellow cap
8	20× concentrated washing buffer	40ml	white cap
9	10× concentrated redissolving solution	50ml	transparent cap

Materials required but not provided

Equipments: microplate reader, printer, homogeniser, nitrogen-drying device, vortex, centrifuge, measuring pipets, balance (a reciprocal sensibility of 0.01 g), incubator
Micropipettors: single-channel 20~200 µL and 100~1000 µL, and multi-channel 30~300 µL ;
Reagents: NaOH, HCl(36%).

Sample pre-treatment

Instructions

1) Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents.
2) Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.
Solution preparation before sample pre-treatment
1) 0.2M HCl: take 17.2ml, add deionized water to 1L;
2) 1M NaOH: dissolve 4 g NaOH in deionized water to 100ml;.
3) Sample diluent: 10×concentrated redissolving solution is mixed with deionized water at 1:9 (1 part 10× concentrated redissolving solution+ 9 parts deionized water).

Samples preparation

a) Tissue (Pork/liver,chicken/ liver etc.)
1) Take 1± 0.05 g of the homogenized tissue sample into 50 mL centrifuge tube, add 2ml 0.2M HCl solution, vortex for 3 min;
2) Then add 400ul 1M NaOH solution and 1.6ml Sample diluent, shake for 3 min, centrifuge at above 4000 r/min at room temperature(20-25 ℃) for 5 min;
3) Take the supernatant, dilute with Sample diluent at 1:2 (50ul supernatant + 100ul Sample diluent), mix for 30s, take 50 µL for analysis.
Fold of dilution of the sample: 15

ELISA procedures

Instructions

1. Bring all reagents and micro-well strips to the room temperature (20-25 ℃).
2. Return all reagents to 2-8 ℃ immediately after use.
3. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA.
4. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

Operation procedures

1. Take out all the necessary reagents from the kit and place at the room temperature (20-25 ℃) for at least 30 min. Note that each reagent must be shaken to mix evenly before use.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8℃, not frozen.
3. Solution preparation: dilute 40 mL of the concentrated washing buffer (20×concentrated) with deionized water at 1:19 (1 part concentrated washing buffer + 19 parts deionized water), or prepare as quantity needed.
4. Numbering: number the micro-wells according to samples and standard preparation; each testing sample and standard solutiond should be performed in duplicate; record their positions.
5. Add 50 µL of the sample or standard solution to separate duplicate wells, and add 50 µL of the antibody working solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25 ℃ for 30 min.
6. Pour the liquid out of the wells, 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 seconds and then flap to dry on absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
7. Add 100 µL of the enzyme conjugate into every well, seal the microplate with the cover membrane, and incubate at 25 ℃ for 30 min, continue as subscribed in 6.

8. Coloration: add 50 µL of the substrate A solution and then 50 µL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15min at dark for coloration.
9. Determination: add 50 µL stop solution into each well. Vortex evenly. Set the wavelength of the microplate reader at 450 nm to determine the OD value.
(we recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).

Result judgment

There are two methods to judge the results; the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the Ampicillinin the sample.

Qualitative determination

The concentration range (ng/mL) can be obtained from comparing the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.3, and that of the sample Ⅱ is 1.0, while those of the standard solutions are as the followings: 2.243 for 0 ppb, 1.816 for 0.1 ppb, 1.415 for 0.3 ppb, 0.74 for 0.9 ppb, 0.313 for 2.7 ppb and 0.155 for 8.1 ppb, accordingly the concentration range of the sample Ⅰ is 2.7 to 8.1 ppb, and that of the sample Ⅱ is 0.3 to 0.9 ppb.

Quantitative determination

The mean values of the absorbance values obtained for the percentage of the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

Percentage of absorbance value = $(B / B_0) \times 100\%$

B—the average OD value of the sample or the standard solution
B_0—the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solutions and the semilogarithmic values of the Ampicillinstandard solutions (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, thus finally obtaining the actual concentration of Ampicillinin the sample.
Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software).

Precautions

1. The room temperature below 25 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value;
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility;
3. Mix every reagent and reaction mixture evenly and wash the microplate thoroughly, otherwise there will be the undesirable reproducibility;
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin;
5. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light;

6. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use;
7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of standard solution 1（0 ppb） of less than 0.5 indicates its degeneration;
8. The optimum reaction temperature is 25 ℃, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

Storage: store at 2-8 ℃, not frozen.
Expiry date: 12 months; date of production is on box.