

GENTAMICIN ELISA TEST KIT
MANUAL

Gentamicin ELISA Test Kit

Catalogue Number. IP100020

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Gentamicin in the sample. The coupling antigen is pre-coated on the micro-well stripes. The Gentamicin in the sample and pre-coated coupling antigen on the micro-well stripes compete for the anti-Gentamicin antibody. 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 the Gentamicin in it. The value is compared to the standard curve and the Gentamicin concentration is subsequently obtained.

Technical specifications

Sensitivity: 0.05 ppb

Incubator temperature: 25°C

Incubator time: 30min~15min

Detection limit: Tissue 2.5 ppb, Liver, milk 3 ppb

Recovery rate: Tissue 90±20%, Liver 80±20%, Milk 90±18%

Cross-reaction rate: Gentamicin 100%, Streptomycin<1%, Dihydrostreptomycin<1%, Neomycin<1%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	5× standard solution (1mL each)	0ppb, 0.05ppb, 0.15ppb, 0.45ppb, 1.35ppb, 4.05ppb	
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	15ml	white cap
9	5× concentrated redissolving solution	50ml	transparent cap
10	10× concentrated extracting solution	50ml	blue cap

Materials required but not provided

Equipments: microplate reader, vortex, centrifuge, homogenizer, measuring pipets, balance (a sensibility reciprocal of 0.01 g).

Micropipettors: single-channel 20-200 µL and 100-1000 µL, and multi-channel 30~300 µL;

Sample pre-treatment

Instructions

1) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;

2) Before the experiment, each experimental utensil must be checked to 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

Dilute the 10× concentrated extracting solution with the deionized water at 1:9 (1 part concentrated extracting solution + 9 parts deionized water)

Samples preparation

a) Tissue (Chicken, pork, fish, shrimp) and Liver(Chicken liver, porcine liver)

1. Take 1 ± 0.05 g of the sample, which already crumble and remove fat, add 5mL diluted extracting solution, shake for 3min; centrifuge at 4000r/min at room temperature (20-25 °C) for 10min.
2. Take 50 μ L supernatant (upper layer), add 450 μ L of the Sample redissolving solution, mix for 30s.
3. Take 50 μ L for analysis.

Fold of dilution of the sample: 50

b) Milk

1. Take 0.5ml homogeneous milk sample, add 2ml diluted extracting solution, shake for 2min; centrifuge at 4000r/min at room temperature (20-25 °C) for 5min.
2. Take 50 μ L supernatant (upper layer), add 450 μ L of the Sample redissolving solution, mix for 30s.
3. Take 50 μ L for analysis.

Fold of dilution of the sample: 50

ELISA procedures

Instructions

1. Bring all reagents and micro-well strips to the room temperature (20-25 °C) before use.
2. Return all reagents to 2-8 °C 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 the kit from the refrigerated environment. Take out all the necessary reagents from the kit and place at the room temperature (20-25 °C) for at least 30 min. Note that each liquid 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 °C, not frozen.
3. Solution preparation: dilute 15mL of the 20X concentrated washing buffer with deionized water at 1:19 (1 part 20X concentrated washing buffer + 19 parts deionized water), or dilute quantity as required.
4. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.
5. Add 50 μ L of the sample or standard solution to separate duplicate wells, and add 50 μ L enzyme conjugate and then 50 μ L 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 °C for 30 min.

6. Pour liquid out of the microwells, add 250 μL /well of diluted washing buffer for 15-30 sec, repeat 4-5 times, then flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
7. Coloration: add 50 μL of the substrate A and then 50 μL of the substrate B into each well. Mix gently by shaking the plate manually, and incubate at 25 $^{\circ}\text{C}$ for 15 min at dark for coloration;
8. Determination: add 50 μL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450nm to determine the OD value (Recommend to read the OD value at the dual-wavelength 450/630nm 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 content of Gentamicin.

Qualitative determination

The concentration range (ng/mL) can be obtained from the comparison 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 0ppb, 1.816 for 0.05ppb, 1.415 for 0.15ppb, 0.74 for 0.45ppb, 0.313 for 1.35ppb and 0.155 for 4.05ppb, accordingly the concentration range of the sample I is 1.35 to 4.05ppb, and that of the sample II is 0.15 to 0.45ppb. (multiplied by the corresponding dilution fold).

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,

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

B—the average OD value of the sample or the standard solution

B₀—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 semilogarithm values of the Gentamicin standard 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 Gentamicin concentration in the sample.

Precautions

1. The room temperature below 25 $^{\circ}\text{C}$ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 $^{\circ}\text{C}$) 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 the 0 standard solution of less than 0.5 indicates its degeneration.
8. The optimum reaction temperature is 25 °C, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

Storage: store at 2-8 °C, not frozen.

Expiry date: 12 months; date of production is on box.