VERSION 1.01

STREPTOMYCIN ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Streptomycin ELISA Test Kit

Catalogue Number. IP100021

Principle

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

Technical specifications

Sensitivity: 0.1 ppb
Incubation Temperature: 37℃
Incubation Time: 30min—30min—15min
Detection limit: Chicken 1 ppb, Chicken liver, milk 4 ppb, Honey, Royal jelly 2 ppb
Recovery rate: Milk 85±22%, Chicken 80±17%, Honey, Royal jelly 75±19%
Cross-reaction rate: Streptomycin 100%, Dihydrostreptomycin 108%, Kalamycin<0.1%, Gentamycin<0.1%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	5× standard solution (1mL each)	0 ppb, 0.1 ppb, 0.4 ppb, 1.6 ppb, 6.4 ppb, 25.6 ppb	
3	Enzyme conjugate	12ml	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	40 ml	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).
Micropipettors: single-channel 20~200 µL, 100~1000 µL; and multi-channel 30～300 µl;
Reagents: H3PO4, NaOH(for honey sample), Acetonitrile(CH3CN), N-hexane, Trichloroacetic acid

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) 0.1 M H3PO4: dissolve 680µL H3PO4 in the deionized water to 100 mL.
2) 0.5% Trichloroacetic acid solution: dissolve 0.5g Trichloroacetic acid in the deionized water to 100 mL
3) 3% Trichloroacetic acid solution: dissolve 3g Trichloroacetic acid in the deionized water to 100 mL
4) 1 M NaOH: dissolve 4 g NaOH in the deionized water to 100 mL.
5) 0.1 M NaOH: dissolve 0.4 g NaOH in the deionized water to 100 mL.
6) The 10×concentrated redissolving solution is diluted with deionized water at 1:9 (1 mL concentrated redissolving solution + 9 mL deionized water)

Samples preparation

a) Milk

1) Take 1 mL milk sample, diluted at 1:39 (1950 µL diluted redissolving solution+ 50 µL milk). Mix for 30 seconds.
2) Take 50 µL for analysis
Fold of dilution of sample: 40

b) Chicken

1) Take 2±0.05 g homogenized sample(remove fat), add 4 mL 3% Trichloroacetic acid. Mix for 2 min.
2) Centrifuge at above 4000 r/min at room temperature for 10 min.
3) Transfer 100 µL supernatant into a new vessel, then add 100µl 0.1M NaOH and 300 µL the diluted redissolving solution, mix properly for 30 seconds.
4) Take 50 µL for analysis.
Fold of dilution of sample: 10

c) Chicken liver

1)Take 2±0.05 g homogenized sample(remove fat), add 6mL 0.5% Trichloroacetic acid and 2ml . CH3CN, mix for 5 min.
2) Centrifuge at above 4000 r/min at room temperature for 10 min.
3) Transfer 2ml supernatant into a new vessel, then add 2ml N-hexane, mix evenly, be static for 3 min, take 0.5ml down-layer bright solution, centrifuge at above 4000 r/min at room temperature for 5min.
4) Take 50µl down-layer bright solution (If there is layering, remove the up-layer, take the down-layer bright solution), add 450µl the diluted redissolving solution, mix for 30s.
5) Take 50 µL for analysis.
Fold of dilution of sample: 40

d) Honey, Royal jelly

1) Weigh 2±0.05 g honey sample, add 4 mL of 0.1 M H3PO4, shake until dissolved fully.
2) Centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 5 min, until liquid is clear (Honey sample can directly to step 3 without centrifuge).
3) Add 900 µL 1 M NaOH, adjust PH to 7-9(For Royal jelly, Transfer the Supernatant to a new vessel).
4) Centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 5 min, until liquid is clear.
5) Take 50 µL suppernatant, add 350 µL of the diluted redissolving solution, mix evenly for 30s.
6) Take 50 µL for further analysis.
Fold of dilution of sample: 20

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.
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. Bring test kit to the room temperature (20-25 ℃) for at least 30 min, note that each reagent must be be shaken evenly before use; put the required micro-well strips into plate frames. Re-sealed the unused microplate, stored at 2-8 ℃, not frozen.
2. Solution preparation: dilute 40 mL of the concentrated washing buffer (20×concentrated) with Deionized water to 800ml;
3. Numbering: number the micro-wells according to samples and standard solution each sample and standard solution should be performed in duplicate record their position.;
4. Add 50 µL of the sample and standard solution to separate duplicate wells, then add 50 µL of antibody working solution to each well, shake properly, seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min;
5. Pour liquid out of microwell, flap to dry on absorbent paper; add 250 µL/well of washing buffer for 15-30 seconds, then take out and flap to dry with absorbent paper, repeat 5 times.
6. Add 100 µL of enzyme conjugate to each well, shake properly, seal the microplate with the cover membrane, and incubate at 37 ℃ for 30 min; continue as step 5 for washing.
7. Coloration: add 50 µL of the substrate A solution, 50 µL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 37 ℃ for 15 min in the 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 450 nm to determine the OD value of every well. (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 Streptomycin

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 0.1ppb, 1.415 for 0.4ppb, 0.74 for 1.6ppb, 0.313 for 6.4ppb and 0.155 for 25.6ppb, accordingly the concentration range of the sample Ⅰis 6.4 to 25.6ppb, and that of the sample Ⅱ is 0.4 to 1.6ppb.

Quantitative determination

The mean values of the absorbance values obtained for the percentage of the average OD value (B) of the testing 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 semilogarithm values of the Streptomycin standard solutions (μg/L) as Y- and X-axis, respectively. Read the corresponding concentration of the testing 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 Streptomycin concentration in 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. So continue to next step immediately after washing.
3. Mix evenly otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. 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.
6. 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.
7. Discard the colouration solution with any 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 37 ℃, 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.