

Streptomycin ELISA Kit

Product #: E6204, 96T

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

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1. Description

Streptomycin belongs to the aminoglycosides family. In veterinary practice, it is used to treat bacterial diseases in cattle, pigs, sheep, and poultry. This current Streptomycin ELISA Kit is based on indirect competitive ELISA to detect Streptomycin residue in cell culture, milk, and honey. In the kit, streptomycin antigen is coated on the microtiter well and samples contained the drug will compete for streptomycin specific antibody with the antigen. After washing and adding the enzyme conjugate, TMB substrate is used to show the reaction color. The concentration of streptomycin 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 Streptomycin residue in cell culture, milk, milk powder and honey. Other applications can also be provided upon request.

3. Kit components

- 1) Microtiter plate, 96wells, 1 plate
- 2) Streptomycin standards, 1mL/vial, 7 vials, 0, 0.05, 0.15, 0.45, 1.35, 4.05 ng/mL and 1ug/mL
- 3) Enzyme conjugate, 1mL, with red cap
- 4) Enzyme conjugate buffer, 10mL, with green cap
- 5) Substrate A, 7mL, with white cap
- 6) Substrate B, 7mL, with red cap
- 7) Stop solution 7 mL, with yellow cap
- 8) 20x Wash buffer, 40mL
- 9) 2x Sample buffer, 50mL

4. Instrument and material required

- 1) ELISA reader, with 450/630nm
- 2) Centrifuge
- 3) Balance, 0.01g
- 4) Centrifuge tube, 2mL, 10mL
- 5) Vortex mixer
- 6) Micropipette, 20-200 μ L, 100-1000 μ L
- 7) Multi-channel pipette, 250 μ L
- 8) graduated pipette, 10mL

5. Reagent required

Deionized water

6. Buffer preparation

Buffer 1: Wash buffer

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

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

Buffer 2: 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 milk sample buffer can be stored at 4°C for 1 month.

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 Cell culture

Please dilute the sample with buffer 1 so that streptomycin is within the standard curve range.

7.3 Milk

take 30 μ L raw milk, mix with 870 μ L sample buffer(**buffer 2**), mix thoroughly, then take 50 μ L for assay.

7.4 Milk powder

Take 1.0 \pm 0.005g sample into 50mL centrifuge tube, add 10mL deionized water, vortex for 5min to dissolve. Then take 100 μ L sample solution and mix with 900 μ L sample buffer (**buffer 2**), vortex for 30s to dissolve, then take 50 μ L for assay.

7.5 Honey

Take 1.0 \pm 0.005g honey sample into 15mL centrifuge tube, add 4mL mix thoroughly by vortex for 5min. Centrifuge for 10min at 3000g, take 300 μ L supernate and then dilute with 300 μ L sample buffer (**buffer 2**), vortex for 30s and then take 50 μ L supernate 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, sample diluent, 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) **Preparation of enzyme conjugate:** dissolve the enzyme conjugate with enzyme conjugate buffer provided in the kit in the ratio of 1:10, for example, 1ml enzyme conjugate + 10ml enzyme conjugate buffer. This solution needs fresh preparation and shall be used immediately after dilution. Calculate the required volume carefully before testing.
- 6) **Add sample/standard/enzyme conjugate solution:** add sample/standard into the wells, 50 μ L per each, then add diluted enzyme conjugate solution, 50 μ L per well, shake gently and then cover the plate and incubate at **25 °C for 30min**.
- 7) **Wash:** take out the plate and pour the liquid out. Use the diluted wash buffer (**buffer 1**) to wash the plate, 250 μ L/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.
- 8) **Coloration:** add substrate A, 50 μ L per well, substrate B, 50 μ L per well, and then cover the plate and incubate **25 °C for 15min**.
- 9) **Stop the reaction:** add stop solution, 50 μ L 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 Qualitative estimation

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

10.2 Quantitative calculation

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

Cell culture: customized

Milk: 30

Milk powder: 100

Honey: 10

12. Specifications of the kit

1) **Sensitivity:** 0.05ng/mL

2) **Specificity:**

streptomycin: 100%;

dihydrostreptomycin 38%.

3) Limit of Detection:

Milk: 1.5 ng/mL

Milk powder: 5ng/mL

Honey: 1ng/mL

4) **Recovery:** 70%-120%

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 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, please 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 25°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.