



Streptomycin ELISA Kit

rev 09/17

(Catalog # E4272-100, 100 assays, Store at 4°C)

I. Introduction:

Streptomycin is an antibiotic (antimycobacterial) drug, the first of a class of drugs called aminoglycosides to be discovered, and it was the first effective treatment for tuberculosis. It is derived from the actinobacterium *Streptomyces griseus*. Streptomycin is a bactericidal antibiotic. Adverse effects of this medicine are ototoxicity, nephrotoxicity, fetal auditory toxicity, and neuromuscular paralysis. BioVision's Streptomycin ELISA kit is a competitive ELISA assay for the quantitative measurement of Streptomycin in tissue, honey and milk. The density of color is proportional to the amount of Streptomycin captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Streptomycin.
Detection Range: 0.1 – 8.1 ppb (ng/ml)
Sensitivity: < 0.1 ppb
Detection limit: 4 ppb for tissue, 2 ppb for honey, 5 ppb for milk and milk powder

III. Sample Type:

Tissue, Honey, Milk and Milk Powder

IV. Kit Contents:

Components	E4272-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4272-100-1
Standard (S1 – S6)	1.0 ml X 6	E4272-100-2-x
High standard (1000 ppb)	1.0 ml	E4272-100-3
Antibody working solution	5.5 ml	E4272-100-4
Enzyme conjugate	11 ml	E4272-100-5
Substrate A solution	6 ml	E4272-100-6
Substrate B solution	6 ml	E4272-100-7
Stop Solution	6 ml	E4272-100-8
Concentrated Wash Solution (20X)	40 ml	E4272-100-9
Concentrated Redissolving solution (5X)	50 ml	E4272-100-10
Plate Sealer	1	E4272-100-11

V. User Supplied Reagents and Equipment:

- Reagents: NaOH, Na₂HPO₄•12H₂O, NaH₂PO₄•2H₂O, N-hexane, Dichloromethane, Acetonitrile, H₃PO₄
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment.

VII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.

Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. Standards: Ready to use

Tube #	S1	S2	S3	S4	S5	S6
Concentration (ng/ml)	0	0.1	0.3	0.9	2.7	8.1

2. **PB buffer:** Dissolve 12.9 g Na₂HPO₄•12H₂O and 2.175 g NaH₂PO₄•2H₂O into deionized water to 100 ml

3. **Redissolving solution:** Dilute the concentrated redissolving solution 5 times with deionized water to be used for sample redissolving, it can be stored at 4 °C environment up to a month.

4. **Wash Buffer:** Dilute 40 ml of the concentrated washing buffer with the distilled or deionized water to 800 ml (or just to the required volume) for using.

5. Sample Preparation:

Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.



- **Tissue:** Weigh 2 g Homogeneous sample into a centrifuge tube, add 8 ml **PB buffer**, oscillate 5min, and incubate for 30 min at water bath at 56°C. Centrifuge at 4000 r/min at room temperature for 10 min. Take 1ml supernatant to another centrifuge tube, add 1 ml **N-hexane**, mix fully, centrifuge at 4000 r/min at room temperature for 5 min. Wipe out the upper N-hexane, take 50 µl Lower water phase, add 450 µl **redissolving solution**, and mix 30s. Use 50µl for the assay. (Dilution times of the sample: 1: 40)
- **Honey:** Weigh 2 g honey sample into centrifuge tube. Add 4 ml 0.04 M H₃PO₄, oscillation until completely dissolved, centrifuge at 4000 rpm at room temperature for 5 min until clear. Add 450 µl **1M NaOH**, adjust to pH 7-9, centrifuge at 4000 rpm at room temperature for 5 min until clear. Take 50 µl supernatant, add 450 µl **redissolving solution**, and mix 30 seconds. Use 50 µl for the assay. (Dilution times of the sample: 1: 20)
- **Milk, Milk powder:** Weigh 2 g samples into centrifuge tube, add 8 ml **PB buffer**, oscillate 5 min, and incubate for 30 min at water bath environment at 56°C. Centrifuge at 4000 rpm at room temperature for 10 min. Wipe out the upper fat, take 50 µl middle clarified liquid, add 450 µl **redissolving solution**, and mix 30 sec. Use 50µl for the assay. (Dilution times of the sample: 1: 50)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. Shake the reagent bottles if there is any crystal. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 50 µl **standards** or **samples** into marked well. Add 50 µl **antibody working solution** into each well.
3. Oscillate the plate for 5 sec, cover the well and incubate for 30 min at RT (25°C).
4. Discard solution, wash plate 5 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (250 µl) using a multi-channel pipette or autowasher. Let it soak for 1 min, and then remove all residual wash-liquid from the wells. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
5. Add 100 µl **Enzyme conjugate** into each well; avoid the light to incubate for 30 min at 25°C.
6. Repeat washing procedure to step 4.
7. Pipette 50 µl **Substrate A solution**, then pipette 50 µl **Substrate B solution** to each well, oscillate gently for 5 sec, avoid the light preservation for 15 min at RT.
8. Add 50 µl **Stop Solution** to each well and oscillate gently to stop the reaction.
9. Read result at 450 nm within 10 minutes.

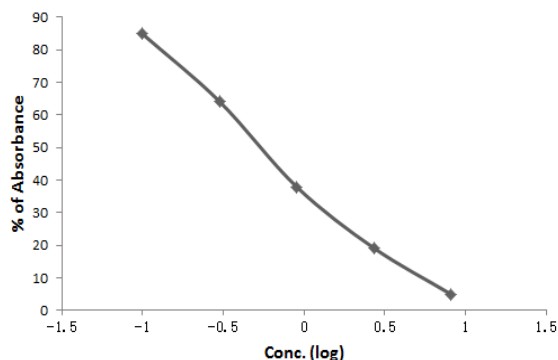
IX. CALCULATION:

Percentage of absorbance value (%) = $A/A_0 \times 100\%$

A: the average (double wells) OD value of the sample or the standard solution; A₀: the average OD value of the 0 ppb standard solution.

To draw the standard curve and calculate, take absorbance percentage of standards as Y-axis, the corresponding log of standards concentration (ppb) as X-axis. Draw the standard semilog curves with X-axis and Y-axis. Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve; last, Multiplied by the corresponding dilution times is the actual concentration of Sal of samples.

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.



X. RELATED PRODUCTS:

- Salbutamol (SALB) ELISA Kit (Cat. No. K4209-100)
- Sulfonamides residue ELISA Kit (Cat. No. K4207-100)
- Aflatoxin B1 (AFB1) ELISA Kit (Cat. No. K4208-100)
- Fluoroquinolones ELISA Kit (Cat. No. K4205-100)
- Gentamicin ELISA Kit (Cat. No. K4206-100)
- Kanamycin ELISA Kit (Cat. No. K4210-100)
- Tetracyclines ELISA Kit (Cat. No. E4273-100)
- Melamine ELISA Kit (Cat. No. E4274-100)