



Neomycin ELISA Kit

rev. 02/20

(Catalog # E4611-100, 100 assays, Store at 4°C)

I. Introduction:

Neomycin is an aminoglycoside antibiotic found in many topical medications such as creams, ointments, and eyedrops. It is typically used as a topical preparation, such as Neosporin. It can also be given orally, where it is usually combined with other antibiotics. Neomycin, like other aminoglycosides, has been shown to be ototoxic causing tinnitus, hearing loss, and vestibular problems in a small number of patients. Standard techniques/instruments (HPLC or GC-MS) are utilized to detect neomycin. However, these techniques are complex, expensive, laborious, and time-consuming. Immunoassay techniques, such as ELISAs are commonly preferred as a simple, reliable and rapid method for the quantification of neomycin in various samples. BioVision's Neomycin ELISA Kit is a competitive-based ELISA that can be used for the determination of this antibiotic in tissue, honey, milk and milk powder.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Neomycin

Detection Range: 0.1 – 8.1 ppb

Sensitivity: 0.1 ppb

Detection limitation: 4 ppb

III. Sample Type:

Tissue (chicken, pork, fish, shrimp), honey, milk, milk powder, liver

IV. Kit Contents:

Components	E4611-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4611-100-1
Standard (S0 – S6)	1 ml X 7	E4611-100-2
Enzyme Conjugate	7 ml	E4611-100-3
Antibody Working Solution	7 ml	E4611-100-4
Substrate A	7 ml	E4611-100-5
Substrate B	7 ml	E4611-100-6
Stop Solution	7 ml	E4611-100-7
Wash Buffer (20X)	15 ml	E4611-100-8
Redissolving Solution (2X)	2X 50 ml	E4611-100-9

V. User Supplied Reagents and Equipment:

- Chemicals: 3% Trichloroacetic acid (TCA), 2% Trichloroacetic acid (TCA), NaOH
- Microplate reader capable of measuring absorbance at 450 nm
- Clean eppendorf tubes and graduated cylinders 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. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.

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

- Redissolving Solution:** mixed Redissolving Solution (2X) with deionized water at 1:1 (1 ml concentrated redissolving solution + 1 ml deionized water)
- Wash Buffer:** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 40 ml of Wash Buffer (20X) into 760 ml deionized or distilled water to prepare 100 ml of Wash Buffer (1X). Keep it at 4°C for one month.
- Standards Concentration:** Ready to Use

Standards	S0	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1	1 ppb (High Control)

4. Sample Preparation:

Note: The prepared sample maybe stored for up to one day at 2-8°C.

A. Tissue(Chicken/liver, pork/liver, shrimp, fish) sample

Take 2 g of the homogenized sample into 50 ml centrifuge tube, add 8 ml 3% Trichloroacetic acid, shake for 5 min, centrifuge at above 4000 r/min at room temperature (20-25°C) for 10 min. Take 2 ml supernatant to another tube, adjust PH to 7.0-7.5 with 2M NaOH (about



100 µl), Dilute it with diluted redissolving solution at 1:4 (100 µl sample + 400 µl diluted redissolving solution), mix for 30s. Take 50 µl for analysis. (Dilution factor: 20)

B. Honey, milk, milk powder sample

Take 2 g sample into 50 ml centrifuge tube, add 8mL 2% Trichloroacetic acid, shake for 5min, centrifuge at above 4000 r/min at room temperature (25°C) for 10 min. Take 2 ml supernatant to another tube, adjust pH to 8.0 with 2M NaOH (about 100 µl). Dilute it with diluted redissolving solution at 1:4 (100 µl sample + 400 µl diluted redissolving solution), mix for 30s. Take 50 µl for analysis. (Dilution factor: 20)

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.

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 of the sample and standard solution to separate duplicate wells; Then 50 µl enzyme conjugate and 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°C for 30 min.
3. 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 sec, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
4. 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°C for 15 min at dark for coloration.
5. Add 50 µl of the stop solution into each well. Mix gently by shaking the plate manually. Read the OD value at the dual-wavelength 450/630nm within 5 min.

IX. CALCULATION:

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbance Value (\%)} = B/B_0 \times 100\%$$

B: The average absorbance value of the sample or standard

B₀: The average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Fluoroquinolones standards solution (ppb) as x-axis. The Fluoroquinolones concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

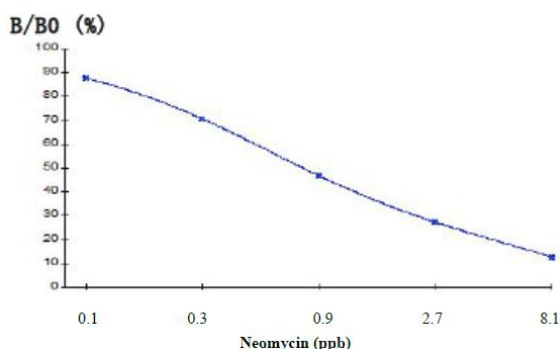


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

- Neomycin Sulfate (Cat. No. 9620)
- Streptomycin ELISA Kit (Cat. No. E4272-100)
- Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
- Kanamycin ELISA Kit (Cat. No. K4210-100)
- Quinolone ELISA Kit (Cat. No. E4530-100)