

Vancomycin ELISA Kit

08/18

(Catalog # E4605-100; 100 assays, Store kit at -20°C)

I. Introduction:

Vancomycin is one of prescribed classes of glycopeptide antibiotics used to treat various bacterial infections including complicated skin infections, bloodstream infections, endocarditis, bone and joint infections, and meningitis. Vancomycin inhibits cell wall synthesis mainly in Gram-positive bacteria by preventing polymerization of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG). Like most other antibiotics, vancomycin has some side-effects such as dizziness, itching, rash, nausea, low blood pressure, muscle pain and fever and it is important to monitor the level of vancomycin in patients. The traditional techniques/instruments (HPLC or GC-MS) for detecting vancomycin are complex, expensive, and time-consuming. Immunoassay techniques, on the other hand, are commonly preferred as a simple, reliable and rapid method. BioVision's Vancomycin ELISA Kit is a competitive-based ELISA that can be used to detect vancomycin in urine and serum samples. This detection kit offers ready-to-use reagents, and can quantify vancomycin (12.8 – 1000 ng/ml) within 120 minutes.

II. Applications:

In vitro, quantitative determination of vancomycin.

Detection Range: 12.8 - 1000 ppb (ng/ml)

Sensitivity: 12.8 ppb

III. Sample Type:

Serum and urine

IV. Kit Contents:

Components	E4605-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4605-100-1
Vancomycin Standard	2 vials	Yellow	E4605-100-2
HRP-conjugate	7 ml	NM	E4605-100-3
Antibody	7 ml	NM/Red	E4605-100-4
TMB substrate	10 ml	Amber	E4605-100-5
Stop Solution	10 ml	NM/Blue	E4605-100-6
Sample Diluent	20 ml	NM	E4605-100-7
Wash Buffer (10X)	50 ml	NM	E4605-100-8
Serum Solution	1.5 ml x 2	Blue	E4605-100-9
Extraction Solution	2 ml	Brown	E4605-100-10
Standard Buffer	20 ml	WM	E4605-100-11
Plate Sealers	4	--	E4605-100-12

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards and sample dilutions

VI. Storage and Handling:

The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at -20°C.

VII. Reagent and Standard Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Serum Solution:** Ready to use. Bring vials to room temperature before use. Aliquot serum solution into multiple vials and avoid freeze-thaw cycles. Store at -20°C.
- **Wash Buffer (10X):** Bring bottle to room temperature. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution is stable at 4°C for one month.
- **Vancomycin Standard:** Add 2.0 ml of Standard Buffer into a vial to prepare 1000 ng/ml (S6). Perform 2-fold dilution to prepare 500 ng/ml (S5). Perform 2.5-fold serial dilutions from S5 (e.g. 400 µl in 600 µl of Standard buffer) to prepare S4 to S1 standards sequentially. S0 contains Standard Buffer only. Prepared standards are stable for 2 weeks at -20°C.

Standards	S0	S1	S2	S3	S4	S5	S6
Concentrations (ppb)	0	12.8	32	80	200	500	1000

VIII. Sample Preparation:

- **Serum**
 1. Add 30 µl of Serum Solution into 270 µl of serum in an Eppendorf tube and vortex well. Incubate samples at 37°C for 45 min.
 2. After the first incubation, incubate samples at 85-90°C for 10 min.
 3. After 10 min, transfer 190 µl of serum to a new Eppendorf tube. Add 10 µl of Extraction Solution into the tube and briefly vortex it.
 4. Spin the tube at 10,000 x g for 10 min and collect the supernatant.
 5. Dilute the supernatant 10 fold using the Sample Diluent. (For example, mix 20 µl of serum with 180 µl of Sample Diluent.)
 6. Use 50 µl per well for the assay.

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Note: Dilution factor: 10

• **Urine**

1. Centrifuge 0.5 ml of urine at 10,000 x g for 5 min and collect the supernatant.
2. Dilute the supernatant 10 fold with Sample Diluent. (For example, mix 20 μ l of urine supernatant with 180 μ l of Sample Diluent.)
3. Use 50 μ l per well for the assay.

Note: Dilution factor: 10

IX. Vancomycin ELISA Assay Protocol:

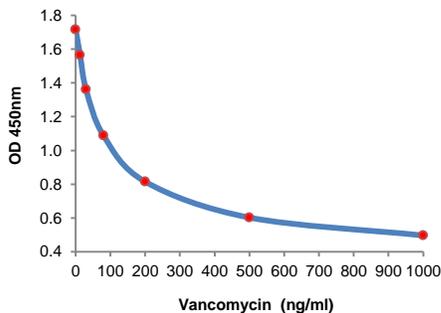
Notes: It is recommended that all standards and samples should be run at least in duplicate. Standard curves must be run each time an assay is performed.

1. Prepare all reagents, standards and samples as described in sections VII and VIII respectively.
2. Add 50 μ l of Standards or Samples per well. Then add 50 μ l of HRP-conjugate and 50 μ l of Antibody to above wells.
3. Cover the plate with a plate sealer and mix well. Incubate the plate at room temperature (25°C) for 90 min.
4. Aspirate all reagents and wash each well 4 times: add 250 μ l of 1X Wash Buffer and incubate for 30 seconds. Remove 1X Wash buffer completely before the next wash. (This is essential for accurate results.) Repeat this step 3 more times.
5. Add 100 μ l of TMB Substrate to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no Vancomycin (S0). When its reading is between 0.75 and 0.9. (usually between 10-30 min after adding the TMB Substrate), add 50 μ l of Stop Solution and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm for the standards and samples within 5 min.

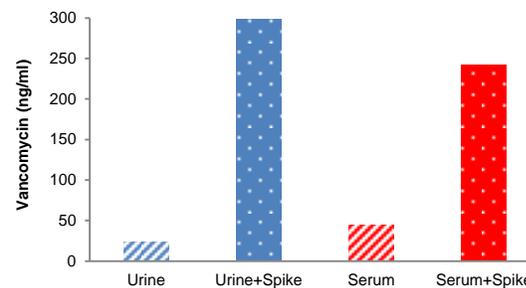
X. Calculation:

The Standard Curve is done by plotting OD 450nm of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of Vancomycin in each sample (ng/ml) can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

A.



B.



Figures. A. Sample standard curve for Vancomycin ELISA Kit (*This standard curve is for demonstration only. A standard curve must be run with each assay*). **B.** Spike recovery experiment: Human serum and urine samples were assayed by spiking approximately 300 ng/ml of vancomycin and showed 80-100% of recovery.

XI. RELATED PRODUCTS:

Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
 Ampicillin ELISA Kit (Cat. No. E4350-100)
 Enrofloxacin (ENR) ELISA Kit (Cat. No. E4277-100)
 Fluoroquinolones ELISA Kit (Cat. No. K4205-100)

Folic Acid ELISA Kit (Cat. No. E4523-100)
 Kanamycin ELISA Kit (Cat. No. K4210-100)
 Quinolone ELISA Kit (Cat. No. E4530-100)

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