ab285230 – Vancomycin ELISA Kit

For the In vitro, quantitative determination of vancomycin
For research use only - not intended for diagnostic use.
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285230

Storage and Stability

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.

Materials Supplied

Item	Quantity	Storage Condition
Vancomycin-BSA Conjugate Coated Plate/ELISA Microplate	8 X 12 Strips	-20°C
Vancomycin Standard	2 vials	-20°C
Goat Anti-Rabbit HRP Conjugate II/HRP-conjugate	7 ml	-20°C
Antibody II/Antibody	7 ml	-20°C
TMB Substrate I/TMB substrate	10 ml	-20°C
Stop Solution VIII/Stop Solution	10 ml	-20°C
Sample Diluent I/Sample Diluent	20 ml	-20°C
10X Wash Buffer II/Wash Buffer (10X)	50 ml	-20°C
Serum Solution I/Serum Solution	1.5 ml x 2	-20°C
Extraction Buffer IV/Extraction Solution	2 ml	-20°C
Standard Buffer II/Standard Buffer	20 ml	-20°C
Microplate Sealing Film/Plate Sealers	4	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 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

Reagent 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 I/Serum Solution: Ready to use. Bring vials to room temperature before use. Aliquot Serum Solution I/serum solution into multiple vials and avoid freezethaw cycles. Store at -20°C.

10X Wash Buffer II/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 II/Wash Buffer by diluting 10 ml of 10X Wash Buffer II/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 II/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 II/Standard buffer) to prepare S4 to S1 standards sequentially. S0 contains Standard Buffer II/Standard Buffer only. Prepared standards are stable for 2 weeks at -20°C.

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

Sample Preparation

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

Urine: Centrifuge 0.5 ml of urine at 10,000 x g for 5 min and collect the supernatant. Dilute the supernatant 10 fold with Sample Diluent I/Sample Diluent. (For example, mix 20 µl of urine supernatant with 180 µl of Sample Diluent I/Sample Diluent). Use 50 µl per well for the assay.
Δ Note: Dilution factor: 10

Assay Protocol

- 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 µl of Standards or Samples per well. Then add 50 µl of Goat Anti-Rabbit HRP Conjugate II/HRP-conjugate and 50 µl of Antibody II/Antibody to above wells.
3. Cover the plate with a Microplate Sealing Film/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 intensively: add 250 µl of 1X Wash Buffer II/Wash Buffer and incubate for 30 seconds. Remove 1X Wash Buffer II/Wash buffer completely before the next wash. (This is essential for accurate results.) Repeat this step 3 more times.
5. Add 100 µl of TMB Substrate I/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 0.5 and 0.6 and the well has changed from medium to dark blue (usually between 5-6 min after adding the TMB Substrate I/TMB Substrate), add 50 µl of Stop Solution VIII/Stop Solution and gently tap the plate to ensure thorough mixing. Do not overdevelop.
7. Measure the OD at 450 nm for the standards and samples within 5 min.

Calculation:

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

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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