

Product Name
Vancomycin-Resistant Enterococcus Gene (vanA, vanB) Detection Kit (PCR Fluorescence)

Specification
12 reactions/kit

Intended Use
This kit uses multiplex Taqman fluorescence quantitative PCR technology for in vitro qualitative detection of *Enterococcus* faecalis ddl gene, *Enterococcus* faecium ddl gene,and vancomycin-resistant *Enterococcus* specific genes *vanA* and *vanB* in human urine samples. The test results can be used for auxiliary diagnosis of vancomycin-resistant Enterococcus infection.
Vancomycin-resistant Enterococcus (VRE) is a common bacterium in the upper respiratory tract, oral cavity or intestine of humans and animals. When it colonizes other mucosal sites, it can cause clinical infections such as endocarditis, with a mortality rate of 21.0% to 27.5%. It is a major nosocomial pathogen worldwide. Among them, the main pathogens in humans are *Enterococcus* faecalis and *Enterococcus* faecium , and the most common drug-resistant genotypes are *vanA* and *vanB*.

Test Principle
The Automated Fully Enclosed qPCR Instrument, uses Intelligent cassette (iCassette) technology to automatically perform nucleic acid extraction, amplification, data reading and result analysis. The instrument includes two parts: nucleic acid extraction and multiple fluorescent PCR. At the same time, the built-in QR code scanner can automatically identify the execution process corresponding to this kit. The uniquely designed software is used to execute the extraction and PCR process of the kit, display the results and analyze the results. The kit contains 12 disposable Galaxy CS VRE iCassettes. The reagents prefilled in the iCassette include nucleic acid extraction reagents and PCR reaction reagents, which can be performed on the Automated Fully Enclosed qPCR Instrument, Since the Automated Fully Enclosed qPCR Instrument is fully enclosed and automatically performs nucleic acid extraction and PCR reactions, it reduces direct cross-contamination of samples. For a complete description of the instrument, please refer to the user manual of the corresponding instrument.
With the specific genes of *Enterococcus* faecalis, *Enterococcus* faecium and vancomycin-resistant *Enterococcus* nucleic acids as the detection targets, this kit designs specific primers and fluorescent probes respectively (*Enterococcus* faecalis ddl gene is labeled with FAM, *Enterococcus* faecium ddl gene is labeled with Texas Red, *vanA* is labeled with CY5.5 and *vanB* is labeled with CY5) for PCR. In the mean time, internal control (HEX labled) is designed to confirmed whether the target strain has been fully treated and whether there are PCR reaction inhibitors.

Components

Component Name			Main ingredients	Quantity
Galaxy CS VRE iCassette (12 pcs)	Nuclei c acid extrac tion reage nt	Magnetic beads	Magnetic microspheres	11 μL/iCassette
		VRE lyophilization A	Proteinase K	1 pc/iCassette
		Internal control lyophilization	Bacillus atrophaeus	1 pc/iCassette
		Virus lysate solution	Guanidinium isothiocyanate	800 μL/iCassette
		Virus binding solution	Guanidinium isothiocyanate	200 μL/iCassette
		Virus washing solution	Sodium chloride	900 μL/iCassette
		Virus eluent	Tris-HCl	90 μL/iCassette
	VRE PCR reage nt	VRE PCR cosolvent solution	5×RT Bufferr	35 μL/iCassette
		VRE lyophilization	Specific primer probe, dNTPs, HiTaq enzyme	1 pc/tube
Control Materials	VRE positive control		Pseudovirus containing target fragment	1 tube (1000μL)
	VRE negative control		Sterilized purified water	1 tube (1000μL)

Note: Components in kits with different batch numbers are not interchangeable.

Storage conditions and Shelf Life
The kit should be stored at 2-8℃ and the shelf life of this kit is 9 months. The transportation temperature range of the kit should be kept at 2-8℃.
Do not open the iCassette lid before adding the sample. If you open the iCassette lid, it must be used within 30 minutes.

Applicable Instrument
Automated Fully Enclosed qPCR Instrument : Galaxy Nano, Galaxy Neo,Galaxy Lite and Galaxy Pro.

Materials Required but Not Provided
• Automated Fully Enclosed qPCR Instrument : Galaxy Nano, Galaxy Neo,Galaxy Lite or Galaxy Pro.
• Ultrasonic machine: Galaxy USL III (*not required for Galaxy Neo*).
• Leak-proof, sterile, screw-capped specimen collection containers.
• Disposable gloves, eye protection, laboratory coats, and labels or permanent marking pen.
• Vortex instrument.
• Pipettes.
• Sterile pipette tips.

Sample Requirements
1. Sample types: Urine
2. Sample collection
Use a sterile disposable urine cup to collect 3 to 5 mL of the midstream urine and sealed it for test.
3. Sample storage and transportation
The samples can be used for testing immediately or stored at 2–8 °C for up to seven days , or stored at ≤ -18℃ for 6 months.The samples should be transported under cooling at 0℃.
4. Biosafety protection principles
All operations should comply with relevant local laws and regulations.

Test Method and Operation
1. Prepare the Galaxy CS VRE iCassette
1.1 Processing samples or control materials in the samples preparation room, vortex the sterile collection tube or quality control for 10 to 15 seconds.
1.2 Open the package of Galaxy CS VRE lyophilization, observe whether the Galaxy CS VRE lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the Galaxy CS VRE lyophilization, and make sure the PCR tube is screwed up.
1.3 Open the lid of the iCassette, and pipette 1000 μl of sample or controls to the Galaxy CS VRE iCassette sample compartment as shown in Figure 1 slowly, close the lid tightly.(Note: After the sample is added to the iCassette, the test needs to start running within 30 minutes)
1.4 Put it into the ultrasonic machine, and sonicate for 20 seconds.
1.5 Place the ultrasonicated iCassette into the instrument.

Note: For the Galaxy Neo, the ultrasound functionality is already built-in, so simply add the sample to be tested or controls into the iCassette and insert it into the machine.



Figure 1. Galaxy CS VRE iCassette (Lateral View)

2. Test Operation
2.1 Turn on the power, press the switch button on front side of the instrument, and the blue light is on, i.e. "the instrument is on". After the instrument is turned on, click the control software icon on the desktop to enter the login interface.
2.2 Log in to the software for the first time with the administrator account (Admin/123456), click "OK" to complete the login.
2.3 Click the "Open" button in the initial interface to open the compartment door of the instrument.
2.4 Put the iCassette that has been added with sample into the instrument and click the "Close" button in the initial interface to close the compartment door. (For multi-channel instrument, iCassette tray holder is required to load the iCassette. Input the sample information at the

corresponding channel position of the control software while loading the iCassette)
2.5 After the compartment door is closed, the instrument scans the corresponding QR code on the iCassette automatically.
2.6 After scanning, click the "Run" button, the software will automatically load the program corresponding to the QR code of the iCassette, and click "OK" to start the program.

2.7 After the program starts , the progress of the instrument running will be displayed in the main interface.
2.8 After the amplification is completed, the compartment door will open automatically.
For the detailed steps of test operation, please refer to user manual of the instrument.

3. Result Analysis
After the experiment is finished, the instrument will automatically save the results, and output the Ct value and amplification curves with results interpretation in the interface.

Quality Control
Internal control: Within the effective range of Ct ≤ 37 for the HEX channel,which is qualified as a valid internal control.
Probe quality control: if the fluorescence signal measurement of the probe displays "probe check fail", it indicates that there is a problem with the probe in the iCassette and the iCassette is unavailable; If the PCR step is performed after the iCassette self-test, it indicates that the iCassette probe is qualified.
The above requirements must be met at the same time in the same experiment, otherwise, this experiment is invalid and needs to be repeated.
Test results of positive control: FAM channel, HEX channel, TEXAS RED channel,CY5 channel and CY5.5 channel are all positive;
Test results of negative control: FAM channel, TEXAS RED channel, CY5 channel and CY5.5 channel are all negative; HEX channel is positive;

Reference Interval
Through the analysis of clinical sample test results, the ROC curve method is used to finally determine the Ct reference value of this kit is 37.

Interpretation of Test Results
The sample to be tested is judged according to the standards in the table below:

FAM Channel	HEX Channel	Texas Red Channel	CY5 Channel	CY5.5 Channel	Result
√	√	×	×	×	<i>Enterococcus</i> faecalis Positive
×	√	√	×	×	<i>Enterococcus</i> faecium Positive
×	√	×	√	×	<i>vanB</i> Gene Positive
×	√	×	×	√	<i>vanA</i> Gene Positive
×	√	×	√	√	<i>vanA</i> and <i>vanB</i> Gene Positive
√	√	×	√	×	<i>Enterococcus</i> faecalis and <i>vanB</i> Gene Positive
√	√	×	×	√	<i>Enterococcus</i> faecalis and <i>vanA</i> Gene Positive
×	√	√	√	×	<i>Enterococcus</i> faecium and <i>vanB</i> Gene Positive
×	√	√	×	√	<i>Enterococcus</i> faecium and <i>vanA</i> Gene Positive
√	√	×	√	√	<i>Enterococcus</i> faecalis, <i>vanA</i> and <i>vanB</i> Gene Positive
×	√	√	√	√	<i>Enterococcus</i> faecium, <i>vanA</i> and <i>vanB</i> Gene Positive
√	√	√	√	√	Positive control
×	√	×	×	×	Negative control
×	×	×	×	×	Invalid

Note:“√”indicates that the result "has an obvious logarithmic amplification curve, and Ct≤37"; “×”indicates the result "no logarithmic amplification curve and Ct>37"; The HEX channel is an internal standard channel. Due to specific competition with the sample, when the test sample is

positive, it may be tested negative.

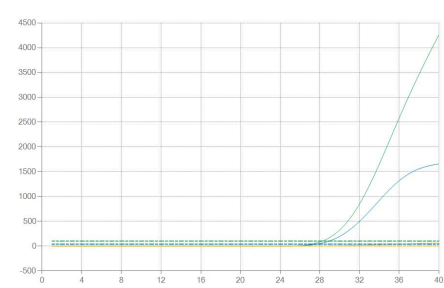


Figure 2. *Enterococcus faecalis* Positive

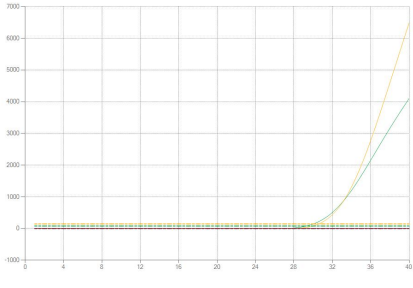


Figure 3. *Enterococcus faecium* Positive

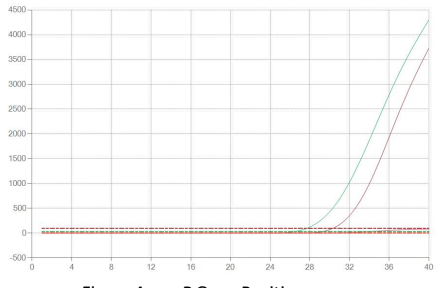


Figure 4. *vanB* Gene Positive

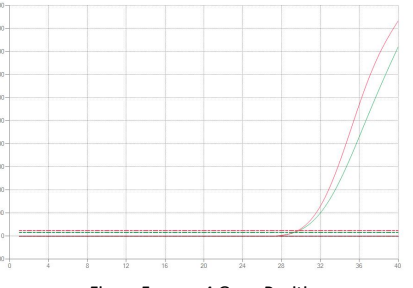


Figure 5. *vanA* Gene Positive

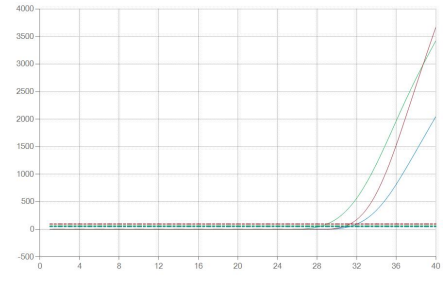


Figure 6. *Enterococcus faecalis* and *vanB* Gene Positive

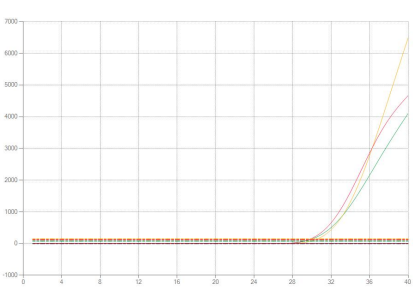


Figure 7. *Enterococcus faecium* and *vanA* Gene Positive

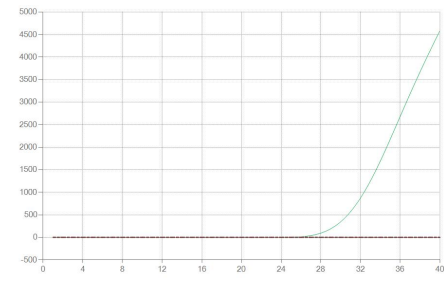


Figure 8. Negative

Limitations of Test Method

1. The test results of the kit are for clinical reference only, and symptoms/signs, medical history, other laboratory tests and treatment response shall be considered comprehensively for clinical diagnosis and treatment of patients.
2. Unreasonable sample collection, transportation and treatment, and too low virus titer in the sample may lead to false negative results.
3. Variations in the target gene sequences of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Ureaplasma urealyticum* or sequence changes caused by other reasons may lead to false negative results.
4. Other unverified interferences, such as endogenous or exogenous substances introduced into the sample, may lead to false positive results;
5. Cross-contamination between samples may lead to false positive results.
6. This kit only detects *vanA* and *vanB* resistance genes and cannot detect other resistance genes;
7. This method only tests urine samples, and the results cannot distinguish between colonizing bacteria, contaminating bacteria, or pathogenic bacteria.

Product performance Index

1. The LoD of the kit

The LoD by the plasmid copy number is 10^3 copies/mL, and the LoD by the bacterial culture method is 10^3 CFU/mL.

Vancomycin-Resistant Enterococcus Gene (*vanA*, *vanB*) Detection Kit (PCR Fluorescence)

2. Analysis specificity

The specificity test results show that there is no cross reaction with *h Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Serratia marcescens*, *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Legionella pneumophila*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*.

3. Precision:

The coefficient of variation of intra-batch precision is $\leq 5\%$.

Precautions

1. The kit must be used within the shelf life.
2. Do not open the iCassette lid before use. If you open it, you must use it within 30 minutes.
3. If you find that the iCassette is oscillated and leaked after adding the sample, do not continue to use it.
4. In order to prevent contamination of the sample, the experimenter should take good care of it and wear disposable gloves and masks.
5. Each iCassette is single-use, please do not reuse it.
6. In order to avoid any potential biological hazards in the sample, the test sample should be regarded as an infectious substance and avoid contact with the skin and mucous membranes; The operation and processing of sample must meet the requirements of local relevant laws and regulations.

References

1. Liu Yang, Zhang Xin, Chen Jianguo, et al. Detection of vancomycin resistance genes in *Enterococcus faecalis* EC-12 [J]. Food and Fermentation Industries, 2018, 44(3): 226-229.
2. Wang Xin, Han Yu, Pan Jiwen, et al. Detection of virulence genes and drug resistance genes of clinically isolated *Enterococcus faecalis* and study of its drug resistance [J]. Acta Tropica Sinica, 2023, 14(2): 138-144. DOI: 10.15886/j.cnki.rdswwb.2023.02.001.
3. KA R IYAMA R, MITSUHATA R, CHOW JW, et al. Simple and reliable multiplex PCR assay for surveillance isolates of vancomycin-resistant enterococci [J]. Journal of Clinical Microbiology, 2000, 38(8): 3 092-3 095.
4. Mai Xinglian, Liu Siyao, Chen Xuan, et al. Study on the correlation between drug resistance and drug resistance genes of *Enterococcus faecalis* in urinary tract infection [J]. Chinese Journal of Clinical Laboratory Management Electronics, 2021, 9(2): 65-70.

Instruction Version

Version: A/1

Date of Issue: May, 2022

Last revised: May, 2025

Symbols

The following symbols may appear on the product labeling:

	In vitro diagnostic medical device		Do not re-use
	Use-by date		Consult instructions for use or consult electronic instructions for use
	Caution		Manufacturer
	Temperature limit		Batch code
	Contains sufficient for <n>		Keep dry
	Keep away from sunlight		Do not use if package is damaged and consult instructions for use
	Date of manufacture		Biological risks
	Catalogue number		CE marking of conformity

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