Vancomycin Enzyme

rp216612

Store at 2-8℃.

Introduction:

Vancomycinase is a water-soluble protein made using recombinant genes. It is a recombinant protein constructed according to the chemical structures of glycopeptide drugs (vancomycin, teicoplanin). The active center of its gene fragment can break the amide bond structures at multiple sites in the structural formula of glycopeptide drugs, causing the drugs to lose their antibacterial properties after ring opening.

It can inactivate the antibacterial properties of vancomycin for injection, teicoplanin, and their oral preparations, thus enabling the positive bacteria in the sterility test to grow well; and making the recovery rate of positive bacteria in the microbial limit test greater than 70%, ensuring the accuracy of the inspection work.

Notes:

1. This product is suitable for extracting proteins from fresh or frozen bacterial and insect cells.
2. This product uses Tris buffer system. Please use the same buffer system for protein purification after extraction.
3. The protein lysis solution obtained from this product can be used for protein quantification using BCA or Bradford method.
4. For special strains, if the extraction effect is not ideal, the sample can be frozen before protein extraction.
5. Depending on the specific situation, protease inhibitors, salts, chelating agents, reducing agents, etc. can be added to this product.

Operation steps:

3.1 Sterility Test

For each batch of vancomycin and teicoplanin samples, use one bottle of vancomycinase (5ml/bottle) and add according to the following table:

Rinsing Solution	1#/500ml	2#/500ml	3#/500ml	4#/500ml	5#/500ml	6#/500ml
Enzyme Addition	\	\	\	\	1ml	1ml
Culture Medium	Thioglycollate Medium 100ml				Tryptic Soy Agar Medium 100ml	
	Positive Control		Bacterial Culture		Mold Culture	
Enzyme Addition	1ml		1ml		1ml	

Add 1ml of the enzyme to each of the 5# and 6# rinsing solutions and shake well. First, moisten the filter membrane of the incubator with the 5# rinsing solution. Pump the dissolved sample solution through the incubator, and rinse the filter membrane of the incubator with the rinsing solutions (1# to 6#) in small amounts for multiple times. Try to avoid any liquid accumulation on the filter membrane. Finally, pump 100ml of the corresponding culture medium into each incubator, add 1ml of vancomycin to each, and shake well.

3.2 Microbial Limit Test

Add 4.5ml of vancomycinase to 1000ml of peptone rinsing solution and shake well. First, moisten the filter membrane with about 50ml of the solution. Pass the dissolved sample solution through the filter membrane, and then rinse it with the peptone rinsing solution containing vancomycinase in small amounts for multiple times, trying to avoid any liquid accumulation on the filter membrane. Take out the filter membrane and place it on a culture medium petri dish. Moisten the entire filter membrane with 0.5ml of vancomycinase for 20 minutes, allowing the enzyme to directly inactivate the trace amount of glycopeptide drugs remaining on the filter membrane, and calculate the recovery rate.

3.3 Verification

Before the work in 3.1 and 3.2, the quality department of the user unit must conduct verification work and keep the verification data. Compile a verification standard SOP according to the results.

4. Inactivation Test

Removing the antibacterial properties of antibiotics can be called neutralization or inactivation. Take 1ml of a 20mg/ml vancomycin or teicoplanin solution and place it in a sterilized empty test tube. Add 1ml of vancomycinase and shake well. Let it stand at room temperature for 20

minutes. Then add 25ml of tryptic soy agar medium, shake it, and add 1ml of Staphylococcus aureus (10^(-6) or 100CFu/ml), and shake well. Incubate at 37°C for 5 days. Shake the test tube every day and observe the growth of bacteria.

Take another test tube with the above operation but without adding 1ml of vancomycinase as the negative control.

The bacteria in the positive tube should grow well within 5 days, and there should be no bacterial growth in the negative tube.