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User's Manual

Human Vancomycin ELISA Kit

REF **DEIANJ11**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This kit can be used in quantitative and qualitative analysis of vancomycin residue in biological samples.

General Description

Vancomycin is an antibiotic used to treat a number of bacterial infections. It is recommended intravenously as a first-line treatment for complicated skin infections, bloodstream infections, endocarditis, bone and joint infections, and meningitis caused by methicillin-resistant S.aureus. Blood levels may be measured to determine the correct dose.

Principles of Testing

This ELISA kit is designed to detect Vancomycin based on the principle of "indirect-competitive" enzyme immunoassay. The microtiter wells are coated with capture BSA-linked antigen. Vancomycin in the sample competes with antigen coated on the microtitre plate for the antibody. After the addition of enzyme conjugate, chromogenic substrate is used and the signal is measured by spectrophotometer. The absorption is inversely proportional to the Vancomycin concentration in the sample.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with coupling antigen
2. Vancomycin standard 1ppm (1 ml)
3. Vancomycin standard 0ppb (1 ml)
4. Vancomycin standard 0.5ppb (1 ml)
5. Vancomycin standard 1.5ppb (1 ml)
6. Vancomycin standard 4.5ppb (1 ml)
7. Vancomycin standard 13.5ppb (1 ml)
8. Vancomycin standard 40.5ppb (1 ml)
9. Sample dilution (50 ml)
10. Antibody solution (7 ml)
11. Enzyme conjugate (12 ml)
12. Substrate (2*6 ml)
13. Stop Solution (7 ml)
14. 20×Wash buffer (50 ml)

Materials Required But Not Supplied

1. Microtiter plate spectrophotometer (450 nm)

2. Constant temperature incubator
3. Shaker
4. Polystyrene centrifuge tube:2 ml, 50 ml
5. Micropipettes: 20 µl-200 µl, 200 µl-1000 µl, 250 µl-multipipette
6. Deionized water

Storage

Store the kit at 2 - 8°C until expiration date.

Specimen Collection And Preparation

Notice and precautions for the users before operation:

- Please use one-off tips in the process of experiment, and change the tips when pipette different reagent.

-Make sure that all experimental instruments are clean, otherwise it will effect the assay result.

Sample Preparation

- Dilute the test sample with the prepared sample diluent to get a final concentration of 0.5-40.5ng/ml (Vancomycin).

- Take 50µl of the prepared solution for assay.

Reagent Preparation

1×wash buffer

Dilute the 20×concentrated wash solution with deionized water in the volume ratio of 1:19 (e.g. 10 ml of 20×concentrated wash solution + 190 ml of deionized water), which will be used for washing the plates. This solution can be stored at 4°C for 1 month.

Assay Procedure

Notice before assay:

1. Make sure all reagents and microwells are all at room temperature (20-25°C).
2. Return all the rest reagents to 2-8°C immediately after used.
3. Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.
4. Avoid the light and cover the microwells during incubation.

Assay Steps

1. Take all reagents out at room temperature (20-25°C) for more than 30min, homogenize before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. The wash solution should be brought to room temperature (20-25°C) before use.
4. Number: Number every microwell position and all standards and samples should be run in duplicate. Record

the standards and samples positions.

5. Add standard solution/sample and antibody solution: Add 50 µl of standard solution or prepared sample to corresponding wells. Add 50 µl of antibody solution to each well, mix gently by shaking the plate manually and incubate for 30 min at 37°C with cover.
6. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 300 µl of diluted wash solution at interval of 10 s for 5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).
7. Add enzyme conjugate: Add 100 µl of enzyme conjugate to each well, mix gently by shaking the plate manually and incubate for 30min at 37°C with cover.
8. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 300 µl of diluted wash solution at interval of 10 s for 5 times. Absorb the residual water with absorbent paper.
9. Coloration: Add 100 µl of substrate solution to each well. Mix gently by shaking the plate manually and incubate for 15 min at 37°C with cover.
10. Measure: Add 50 µl of the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450-620 nm. (Read the result within 5min after addition of stop solution.)

Calculation

Percentage absorbance

The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

Absorbance (%) = (B/B0) * 100%

B ——absorbance of standards or samples

B0 ——absorbance of zero standard (0 ng/ml)

Specificity

Vancomycin

Linearity

0.5-40.5 ng/ml

Precautions

1. The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25°C).
2. Do not allow microwells to be dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.
3. Mix the homogenate and elute the plate adequately.
4. Avoid the stop solution touching skin for the 2M H_2SO_4

5. Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
6. Storage constitution: Keep the ELISA kits at 2-8°C without frozen. Avoid direct sunlight during all incubations. Covering the microtiter plates is recommended.
7. The reagents go bad: Substrate solution should be abandoned if its color has changed. The reagents may be turn bad if the absorbance value of the zero standard is less than 0.5 (A450nm<0.5).

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