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Bevacizumab Quantitation Kit (ELISA)

【Generic Name】

Bevacizumab Quantitation Kit (ELISA)
Cat#: 17-0095

【Intended Use】

To detect the contents of Bevacizumab antibody in the blood samples.

【Principle】

This product adopts the principle of Double Antibody Sandwich Method (Sandwich Elisa). The flat-bottom 96-well plates are coated with anti-Bevacizumab antibody. After adding the samples, the anti-Bevacizumab monoclonal antibody labeled with HRP is used for detection. The content of the Bevacizumab antibody in the blood samples can be detected by the degree of TMB color development.

【Materials and Reagents】

1. Coated plate, 12 wells × 8 strips
2. Enzyme conjugate, 120 μ L×1 tube (diluted 100 times for use)
3. BSA, 3G ×1 pack
4. Calf serum, 5ml×1 vial
5. 20× Washing Buffer, 50mL×1 vial
6. 20× PBST Washing Buffer, 50mL×1 vial
7. Substrate Solution A, 7mL×1 vial
8. Substrate Solution B, 7mL×1 vial
9. Termination Solution, 7mL×1 vial
10. Sealing plate film, 2 pieces
11. Instruction book

【Storage】

1. All components remain stable under the condition of 2-8℃;
2. Avoid light. Valid for six months.

【Protocol for Detection】

1. Equilibration
   Equilibrate the required reagents at room temperature (18~25℃) for 30 minutes.
2. Dosing solution: Please configure the reagents before use.

2.1 1×washing buffer: Take 1 vial of 20×PBST washing buffer, dilute it to 1000ml with deionized water, mix well.

2.2 1×dilution buffer: Take 1 vial of 20×washing buffer, dilute it to 1000ml with deionized water, mix well.

2.3 Sample dilution buffer: After Calculating the total volume of the sample dilution buffer, dilute the calf serum 5 times using the prepared 1×dilution buffer (step 2.2), mix well.

2.4 Enzyme dilution buffer: Dissolve BSA (3g/pack) completely into 100ml of the prepared 1×dilution buffer (step 2.2), mix well.

2.5 Enzyme solution: Dilute the enzyme conjugate 100 times with the enzyme dilution buffer prepared in step 2.4, mix well.

2.6 Standards: Dilute the Bevacizumab by pure monkey negative serum by grads' multiple. the gradients are 102400ng/ml, 51200ng/ml, 25600ng/ml, 12800ng/ml, 6400ng/ml, 3200ng/ml, 1600ng/ml, 800ng/ml, 400ng/ml, 200ng/ml and 100ng/ml. Dilute each gradient twice by the sample dilution buffer (step 2.3) respectively, mix well.

3. Adding standards
   Remove the coated plate from the sealed bag and dilute the standard to the different concentrations. After adding 100μl of standard or sample to each well (including negative control), seal the plate with sealing film. Place the plate in a shaking incubator (37°C, 200 rpm) and incubate for 120 minutes
4. Washing
   Discard the liquid in each well, fill the microwells (350μl/well) with 1× washing buffer, and discard the liquid in the wells after 30 seconds. Repeat these steps for 3 times, then pat the plate on the paper towel after the last wash.
5. Adding enzyme solution
   After diluting the enzyme conjugate 100 times with the enzyme solution buffer, add the solution to the microplate (100μl per well). Seal the plate with sealing film. Place the plate in a shaking incubator (37°C, 200 rpm) and incubate for 60 minutes.
6. Washing
   Repeat step 4.
7. Coloring
   Add 50μl each of Substrate Coloring Solution A and B into each well. Mix well with gentle tapping. Then incubate the plate at room temperature for 10 minutes in the dark.
8. Termination
   Terminate the reaction by adding 50μl of 0.2M H_2SO_4 into each well and mix gently. Set the main wavelength of the microplate reader at 450nm and the reference wavelength at 630nm. Measure the absorbance (OD value) of each well.

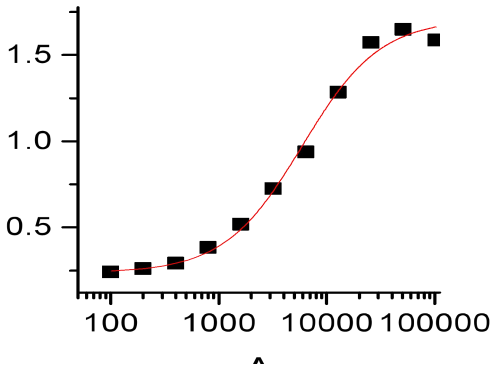
【Data Analysis】

It is recommended to adopt the fitting method of double logarithm for fitting and calculation.

Standard Curve Concentration	OD Value		Mean Value
102400	1.497	1.675	1.586

51200	1. 857	1. 441	1. 649
25600	1. 546	1. 6	1. 573
12800	1. 287	1. 283	1. 285
6400	0. 95	0. 926	0. 938
3200	0. 774	0. 676	0. 725
1600	0. 505	0. 53	0. 5175
800	0. 39	0. 377	0. 3835
400	0. 287	0. 299	0. 293
200	0. 259	0. 263	0. 261
100	0. 241	0. 241	0. 241
0	0. 199	0. 219	0. 209

After double logarithm fitting of the concentrations and OD values, the curve equation is as follows:



R^2=0.99199
A_1=0.23664
A_2=1.70915
X_0=5915
P=1.20581

【Product Performance Index】

1. Linear range:100～102400ng/ml
2. Detection limit: <200ng/ml
3. Accuracy：CV% ≤15% (n=10)

【Limitations】

1. This kit is only used to detect the content of Bevacizumab antibody in blood samples.
2. Results out of the measurement range of the kit are unreliable.
3. Severe hemolysis, chyle, and bilirubin samples may cause abnormal test results.

【Caution】

1. Avoid cross contamination.
2. Follow reader measure as a standard.
3. All samples and buffers should be added or removed with pipette.
4. Do not mix reagents from different batches.