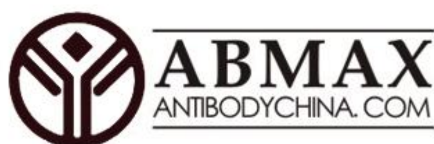

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DM1- Antibody-Drug Conjugate (ADC) Quantitation Kit (ELISA)

【Generic Name】

DM1- Antibody-Drug Conjugate (ADC) Quantitation Kit (ELISA)
Cat#: 17-0085

【Intended Use】

To detect the contents of DM1-ADC 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-DM1 antibody. After adding the samples, the human IgG monoclonal antibody labeled with HRP is used for the detection. The content of the DM1-ADC in the samples can be detected by the degree of TMB color development.

【Materials and Reagents】

1. Coated plate, 12 wells × 8 strips, (Cat:17-0085a)
2. 20× Washing Buffer, 50mL×1 vial, (Cat:17-0085b)
3. Enzyme dilution buffer, 12mL ×1 vial, (Cat:17-0085c)
4. Enzyme conjugate, 120 μ L×1 tube, (Cat:17-0085d)
5. Sample dilution buffer ,30mL×1 vial, (Cat:17-0085e)
6. Substrate Solution A, 7mL×1 vial, (SI00192)
7. Substrate Solution B, 7mL×1 vial, (SI00193)
8. Termination Solution, 7mL×1 vial, (SI00194)
9. Sealing plate film, 2 pieces
10. Instruction book

【Storage】

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

【Necessary reagents which not prepared】

Standards: DM1-ADC. Dilute by sample dilution buffer. The suggested dilution gradient is 1 ng/mL, 3 ng/mL, 10 ng/mL, 30 ng/mL, 100 ng/mL, 300 ng/mL, and 1000ng/mL.

【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×washing buffer, dilute it to 1000ml with deionized water, mix well.

2.2 Sample diluting: Dilute the sample 500 times by the sample dilution buffer, mix well.

2.3 Enzyme solution: Dilute the enzyme conjugate 100 times by the enzyme dilution buffer, mix well.

2.4 Standard dilution buffer: Dilute human serum by sample dilution buffer (the proportion of human serum in the mixture should be 0.2%).

2.5 Standards: Dilute the DM1-ADC by the standards dilution buffer (prepared in step 2.4) by grads' multiple. The gradients are 1ng/ml, 3ng/ml, 10ng/ml, 30ng/ml, 100ng/ml, 300ng/ml, and 1000ng/ml.
If the sample concentrations are too high, the dilution ratio could be increased. Meanwhile, the human serum added in the sample dilution buffer also need to be added by folds.

3. Adding standards
   After removing the coated plate from the sealed bag, add 100μl of diluted sample to each well. Meanwhile, the negative control (sample dilution buffer and the negative serum) should also be set.
4. Incubation: Seal the plate with sealing film and place it in a shaking incubator (37°C, 200 rpm) for 90 minutes.
5. 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.
6. Adding enzyme solution
   Adding 100ml enzyme 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.
7. 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 5 times, then pat the plate on the paper towel after the last wash.
8. 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 5~10 minutes in the dark.
9. 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】

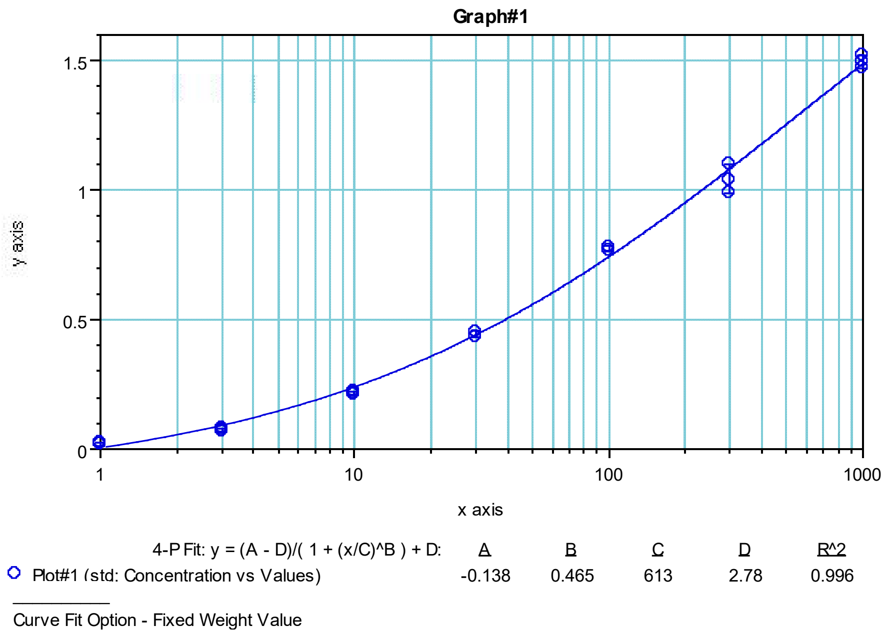
1. Quantitative result determination
   Cutoff value=N+2*SD. (N means the average OD value, calculated as 0.05 when smaller than 0.05. SD

means the standard deviation of the negative serum)

When the OD value is smaller than the cutoff value, the result is negative. Otherwise, the result is positive.

2. Calculation

It is recommended to adopt the fitting method of four parameters for fitting and calculation.



【Product Performance Index】

1. Sensitivity: ≤3ng/ml
2. Linear range:1～1000ng/ml
3. Accuracy：CV% ≤15% (n=10)

【Limitations】

1. This kit is only used to detect the content of DM1-ADC 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.