Version 21-04/24

User's Manual

Human CD56(Neural Cell Adhesion Molecule) ELISA Kit

REF **DEIA3974**

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

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PRODUCT INFORMATION

Intended Use

CD56 ELISA Kit allows for the in vitro quantitative determination of CD56 concentrations in serum, plasma, tissue homo

Principles of Testing

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre-coated o biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Strepta enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding a absorbance at 450nm in a microplate reader, and then the concentration of target can be calculated.

Reagents And Materials Provided

No.	Item	Specifica
E001	ELISA Microplate(Dismountable)	8×12
E002	Lyophilized Standard	2vial
E039	Sample/Standard Dilution Buffer	20ml
E003	Biotin-labeled Antibody(Concentrated)	120ul
E040	Antibody Dilution Buffer	10ml
E034	HRP-Streptavidin Conjugate(SABC)	120ul
E049	SABC Dilution Buffer	10ml
E024	TMB Substrate	10ml
E026	Stop Solution	10ml
E038	Wash Buffer(25X)	30ml
E006	Plate Sealer	5pieces
E007	Product Description	1copy

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the ex

Standard(n=5)	37°C for 1 month
Average (%)	80

To minimize extra influence on performance, operation procedures and lab conditions, especially room temperature, air whole assay from the beginning to the end.

Materials Required But Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and di
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Storage

2-8°C for 6 months

Specimen Collection And Preparation

Serum: Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma using EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g cholesterol samples.

Tissue Homogenates: As hemolysis blood has relation to assay result, it is necessary to remove residual blood by was volume depends on the weight of the tissue. Normal, 9mL PBS would be appropriate to 1 gram tissue pieces. Some pro sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then the total protein concentration of each pore sample should not exceed 0.3mg.

Cell Culture Supernatant: Centrifuge supernatant for 20 minutes at 1000×g at 2-8°C to remove insoluble impurity and

Cell Culture Lysate: Commercial RIPA kits are recommended to follow the instructions provided. Generally, 0.5ml RIP by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.

Other Biological Fluids: Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the

Note: Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at -20°C (assay thaw cycles. The hemolytic samples are not suitable for this assay.

Sample Dilution

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to ma dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And als with PBS first and then dilute the samples with Sample Dilution.

The matrix components in the sample will affect the test results, which it need to be diluted at least 1/2 with Sa

Reagent Preparation

Bring all reagents and samples to room temperature for 20 minutes before use.

Wash Buffer

If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not excee temperature before use.

Dilute 30ml Concentrated Wash Buffer into 750ml Wash Buffer with deionized or distilled water. Put unused solution ba

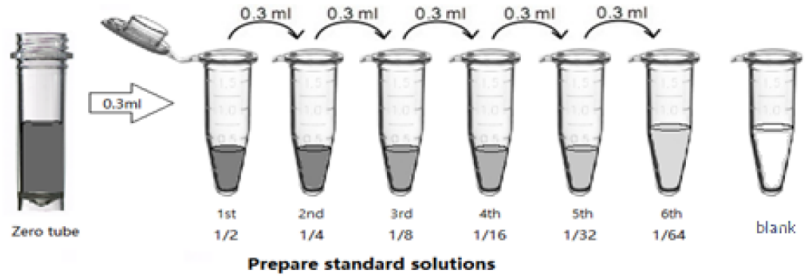
Standards

1) Add 1 ml Sample Dilution Buffer into one Standa temperature for 10 minutes and mix them thorou

Note: If the standard tube concentration higher than the range of the kit, please dilute it and labeled as zero tube.

2) Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1 Dilution Buffer into each tube. Add 0.3ml of the a mix them thoroughly. Transfer 0.3ml from 1st tub 2nd tube to 3rd tube and mix them thoroughly, ar control.

Note: It is best to use Standard Solutions within 2 hours.



Prepare standard solutions

Preparation of Biotin-labeled Antibody Working Solution

Prepare it within 1 hour before experiment.

1) Calculate required total volume of the working so than the total volume.)
2) Dilute the Biotin-detection antibody with Antibody 1ul Biotin-labeled antibody into 99ul Antibody Dil

Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution

Prepare it within 30 minutes before experiment.

1) Calculate required total volume of the working so

than the total volume.)

2) Dilute the SABC with SABC Dilution Buffer at 1:1 99ul of SABC Dilution Buffer.)

Assay Procedure

Washing Notes

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or oth from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automatic: Aspirate all wells, and then wash plate with 350ul wash buffer. After the final wash, invert plate, and clap th minute. (**Note:** set the height of the needles; be sure the fluid can be sipped up completely)

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilib

1. Set standard, **test samples (diluted at least 1/2** pre-coated plate respectively, and then, records standard and sample in duplicate. **Wash plate 2 (blank) wells!**
2. **Prepare Standards:** Aliquot 100ul of zero tube, Sample Dilution Buffer (blank) into the standard
3. **Add Samples:** Add 100ul of properly diluted sar
4. **Incubate:** Seal the plate with a cover and incuba
5. **Wash:** Remove the cover and discard the plate let the wells dry completely at any time.
6. **Biotin-labeled Antibody:** Add 100ul Biotin-labe test sample and blank wells). Add the solution at cover the plate and incubate at 37°C for 60 minu
7. **Wash:** Remove the cover, and wash plate 3 time wells for 1-2 minutes each time.
8. **HRP-Streptavidin Conjugate (SABC):** Add 100 and incubate at 37°C for 30 minutes.
9. **Wash:** Remove the cover and wash plate 5 time for 1-2 minutes each time.
10. **TMB Substrate:** Add 90ul TMB Substrate into e 10-20 minutes. (**Note:** The reaction time can be change, but not more than 30 minutes. You can standard wells.)
11. **Stop:** Add 50ul Stop Solution into each well. The Stop Solution should be as the same as the TMB
12. **OD Measurement:** Read the O.D. absorbance a stop solution.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtai

Summary

Step1: Wash plate 2 times before adding Standard, Sample (diluted at least 1/2 with Sample Dilution Buffer) and Contr

Step2: Add 100ul standard or sample to each well and incubate for 90 minutes at 37°C.

Wash step: Aspirate and wash plates 2 times.

Step3: Add 100ul Biotin-labeled antibody working solution to each well and incubate for 60 minutes at 37°C.

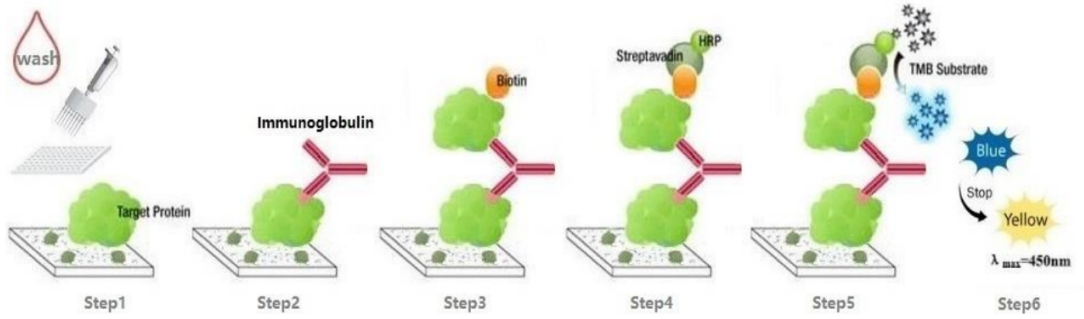
Wash step: Aspirate and wash plates 3 times.

Step4: Add 100ul SABC Working Solution into each well and incubate for 30 minutes at 37°C.

Wash step: Aspirate and wash plates 5 times.

Step5: Add 90ul TMB Substrate Solution. Incubate 10-20 minutes at 37°C.

Step6: Add 50ul Stop Solution. Read at 450nm immediately and calculation.



Calculation

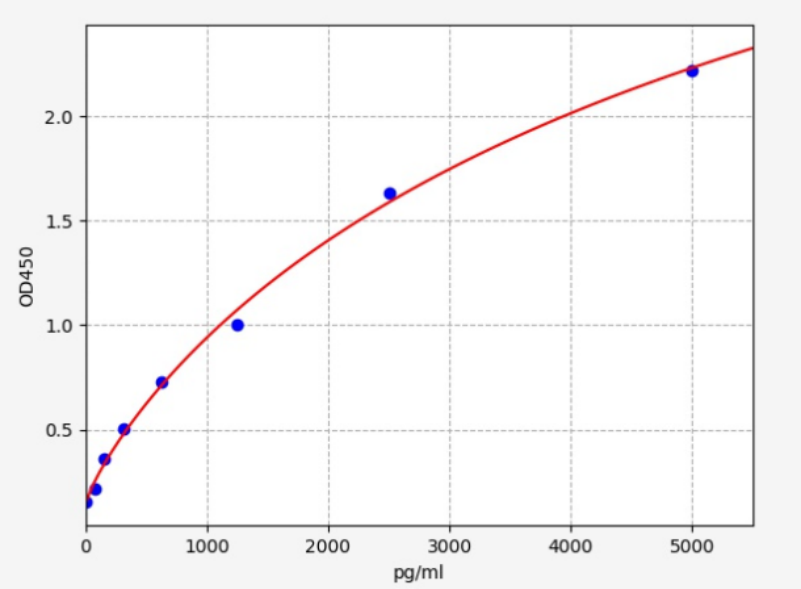
(the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well)

The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) *vs.* the respective concentratio

The target concentration of the samples can be interpolated from the standard curve.

It is recommended to use some professional software to do this calculation.

Typical Standard Curve



Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Detection Range

78.125-5000pg/ml

Sensitivity

< 46.875pg/ml

Specificity

This assay has high sensitivity and excellent specificity for detection of CD56. No significant cross-reactivity or interfere

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between CD

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of CD56 and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8
serum(n=5)	88-104%	85-103%	86-105%
EDTA plasma(n=5)	83-99%	85-99%	82-97%
heparin plasma(n=5)	82-98%	82-99%	81-87%

Recovery

Matrices listed below were spiked with certain level of CD56 and the recovery rates were calculated by comparing the measured value to the expected amount of CD56 in samples.

Matrix	Recovery range(%)	Average(%)
serum(n=5)	87-99	94
EDTA plasma(n=5)	93-105	99
heparin plasma(n=5)	95-103	99

Precautions

1. To inspect the validity of experiment operation a
   experiment using standards and a small number
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down a
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully was
6. Duplicate well assay is recommended for both st
7. Don't let microplate dry at the assay, for dry plate
8. Don't reuse tips and tubes to avoid cross contam
9. Avoid using the reagents from different batches