Version 24-04/25

User's Manual

Anti-CD28 antibody ELISA Kits

REF **DEIA-BN2310-2**

Σ **96T**

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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

The kit is developed for the detection of anti-CD28 antibody in Bioprocess manufacturing applications. It is used as a tool to aid in routine quality control of in-process streams as well as final product.

It is for research use only.

General Description

CD28 antibodies can be used to stimulate the proliferation and activation of T cells in CIK cell therapy. Under the cooperation of other cytokines, such as IL2 and IL1a, CIK cells with rapid proliferation, high tumoricidal activity, broad tumor killing spectrum and non-MHC-restricted tumor killing characteristics are generated,which has significant effects on the treatment of cancer, chronic leukemia, liver disease and neurological diseases. Obviously, it is necessary to control the residues of raw materials in the final cell therapy products.

Principles of Testing

This assay kit is used to measure the titer of Anti-CD28 Antibody by employing an indirect ELISA. Immobilize Human CD3E & CD3G on the microplate. Then add the samples, incubate and wash the wells. Next add Secondary antibody HRP-Anti-Mouse IgG to the plate, incubate and wash the wells. Lastly load the substrate into the wells and monitor color development in proportion with the amount of antibody present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of antibody bound.

Reagents And Materials Provided

1. Pre-coated Human CD28 Microplate, 1 plate, Solid, Unopened 2-8°C, Opened 2-8°C
2. Anti-CD28 Antibody Standard, 20 µg, Powder. Unopened 2-8°C, Opened -70°C
3. HRP-Goat anti-Mouse IgG, 10 µg, Powder, Unopened 2-8°C, Opened -70°C
4. 10×Washing Buffer, 50 mL, Liquid, Unopened 2-8°C, Opened 2-8°C
5. 2×Dilution Buffer, 50 mL, Liquid, Unopened 2-8°C, Opened 2-8°C
6. Substrate Solution, 12 mL, Liquid, Unopened 2-8°C, avoid light. Opened 2-8°C, avoid light
7. Stop Solution, 7 mL, Liquid, Unopened 2-8°C, Opened 2-8°C

Materials Required But Not Supplied

Single or multi-channel micropipettes and pipette tips: need to meet 10 µL, 300 µL, 1000 µL injection requirements;

37° C Incubator;

Single or dual wavelength microplate reader with 450nm and 630nm filter;

Tubes: 1.5mL,10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

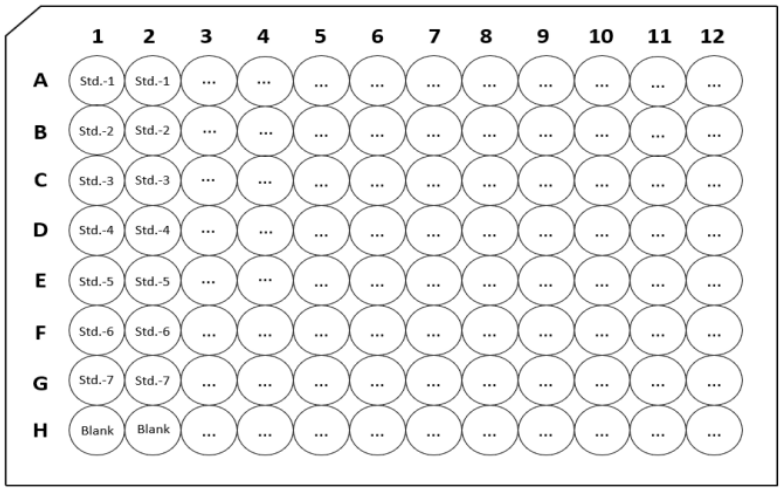
Storage

1. The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.
2. The opened kit should be stored per TABLE 1. The shelf life is 30 days from the date of opening.

Note:

1. Do not use reagents past their expiration date.
2. Find the expiration date on the outside packaging.

Plate Preparation



Note: Blank is a Blank Dilution Buffer hole.

Reagent Preparation

Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, place the sample in a 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use. Reconstitute the provided lyophilized materials to stock solutions with distilled, sterile water as recommended in Table and place the materials for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking. The reconstituted stock solutions should be stored at -70°C. It is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 5μg.

Note: Considering innevitable minor quantatition variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for even better accuracy.

Table. Reconstitution methods

Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
Anti-CD28 Antibody Standard	20 µg	100 ug/mL	200 µL water
HRP-Goat anti-Mouse IgG	10 µg	100 ug/mL	100 µL water

Assay Procedure

1. Working fluid preparation

a. Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

b. Preparation of 1×Dilution Buffer:

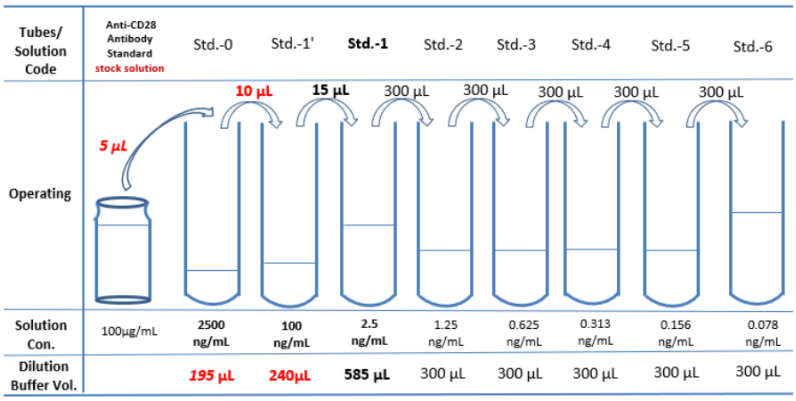
Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

c. Preparation of HRP-Goat anti-Mouse IgG working fluid:

Dilute HRP-Goat anti-Mouse IgG to 0.08 µg/mL with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

2. Preparation of Standard curve

Make serial dilutions of the Anti-CD28 Antibody Standard as a Standard curve with Dilution Buffer as recommended in Figure.



3. Add Samples

Add 100µL serially diluted Anti-CD28 Antibody Standard curve and samples to each well. For blank Control wells, please add 100µL 1×Dilution Buffer. Seal the plate with microplate sealing film and incubate at 37°C for 1 hour.

4. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, gently tap the plate for 1 min, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

5. Add HRP-Goat anti-Mouse IgG

For all wells, add 100 µL HRP-Goat anti-Mouse IgG (dilute to 0.08 µg/mL) working solution. Seal the plate with microplate sealing film and incubate at 37°C for 1 hour.

6. Washing

Repeat step 4.

7. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at 37°C for 20 min, avoid light.

8. Termination

Add 50 µL Stop Solution to each well, and tap the plate gently for 5 min to allow thorough mixing. **Note: the color in the wells should change from blue to yellow.**

9. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer.

Note: To reduce the background noise, subtract the value read at OD450 nm with the value read at OD630 nm.

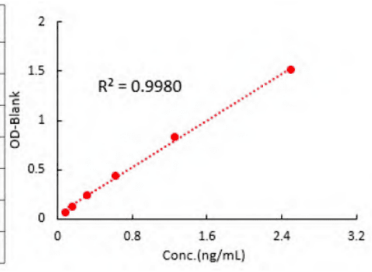
Calculation

1. Normal range of Standard curve: $R^2 \geq 0.9900$, detection range: 0.078-2.5 ng/mL.
2. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.
3. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted from the OD value of the blank control. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Linear regression equation or Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

Typical Standard Curve

The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Anti-CD28 Antibody (ng/mL)	OD450-630nm	OD450-630nm-Blank
2.5	1.535	1.515
1.25	0.848	0.828
0.625	0.455	0.435
0.3125	0.258	0.238
0.15625	0.146	0.126
0.078125	0.084	0.064
Blank	0.020	0.000



Precautions

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. All reagents should be balance to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, warm to room temperature until the crystals have completely dissolved.

5. The kit should be stored at 2°C to 8°C.

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