A014-EN.02

resDetect™ Anti-CD28 Antibody ELISA Kit

(Enzyme-Linked Immunosorbent Assay)

Catalog Number：CRS-A014

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

US and Canada:	**Tel**: +1 800-810-0816	**Web**: http://www.acrobiosystems.com
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INTENDED USE

The kit is developed for the detection of anti-CD28 antibody in Bioprocess manufacturing applications. It is intended for research use only (RUO).

BACKGROUND

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.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the titer of Anti-CD28 Antibody by employing an indirect ELISA. Immobilize Human CD28 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.

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. Bring all reagents and samples to room temperature (20℃-25℃) 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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MATERIALS PROVIDED

Table 1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
CRS014-C01	Pre-coated Human CD28 Microplate	1 plate	Solid	2-8°C	2-8°C
CRS014-C02	Anti-CD28 Antibody Standard	20 μg	Powder	2-8°C	-70°C
CRS014-C03	HRP-Goat anti-Mouse IgG	10 μg	Powder	2-8°C	-70°C
CRS014-C04	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
CRS014-C05	2xDilution Buffer	50 mL	Liquid	2-8°C	2-8°C
CRS014-C06	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
CRS014-C07	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

STORAGE AND EXPIRATION DATA

Unopened kit should be stored at 2°C -8°C upon receiving.

Find the expiration date on the outside packaging and do not use reagents past their expiration date.

The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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

37ºC Incubator;

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

Tubes: 1.5mL,10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

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REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in a 37ºC 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 2 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℃. It is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 5 μg.

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 2. Reconstitution methods

ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
CRS014-C02	Anti-CD28 Antibody Standard	20 μg	100 μg/mL	200 μL water
CRS014-C03	HRP-Goat anti-Mouse IgG	10 μg	100 μg/mL	100 μL water

RECOMMENDED SAMPLE PREPARATION

1. Working fluid preparation

1.1 Preparation of 1×Washing Buffer:

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

1.2 Preparation of 1×Dilution Buffer:

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

1.3 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.

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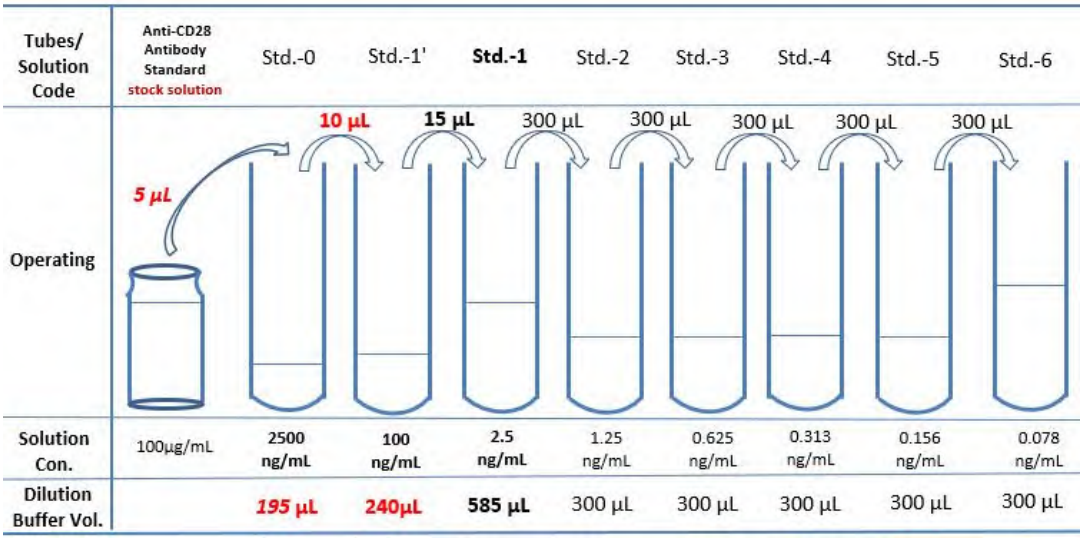
2. Preparation of Standard curve

Make serial dilutions of the Anti-CD28 Antibody Standard as a Standard curve with Dilution Buffer as recommended in Figure 1.

Figure 1. Preparation of 1:1 serial dilutions of the Anti-CD28 Antibody



3. Add Samples

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

6. Washing

Repeat step 4.

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7. Substrate Reaction

Add 100 µL **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at 37℃ 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* $OD_{450\ nm}$ *with the value read at* $OD_{630\ nm}$.

CALCULATION OF RESULTS

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 to 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.

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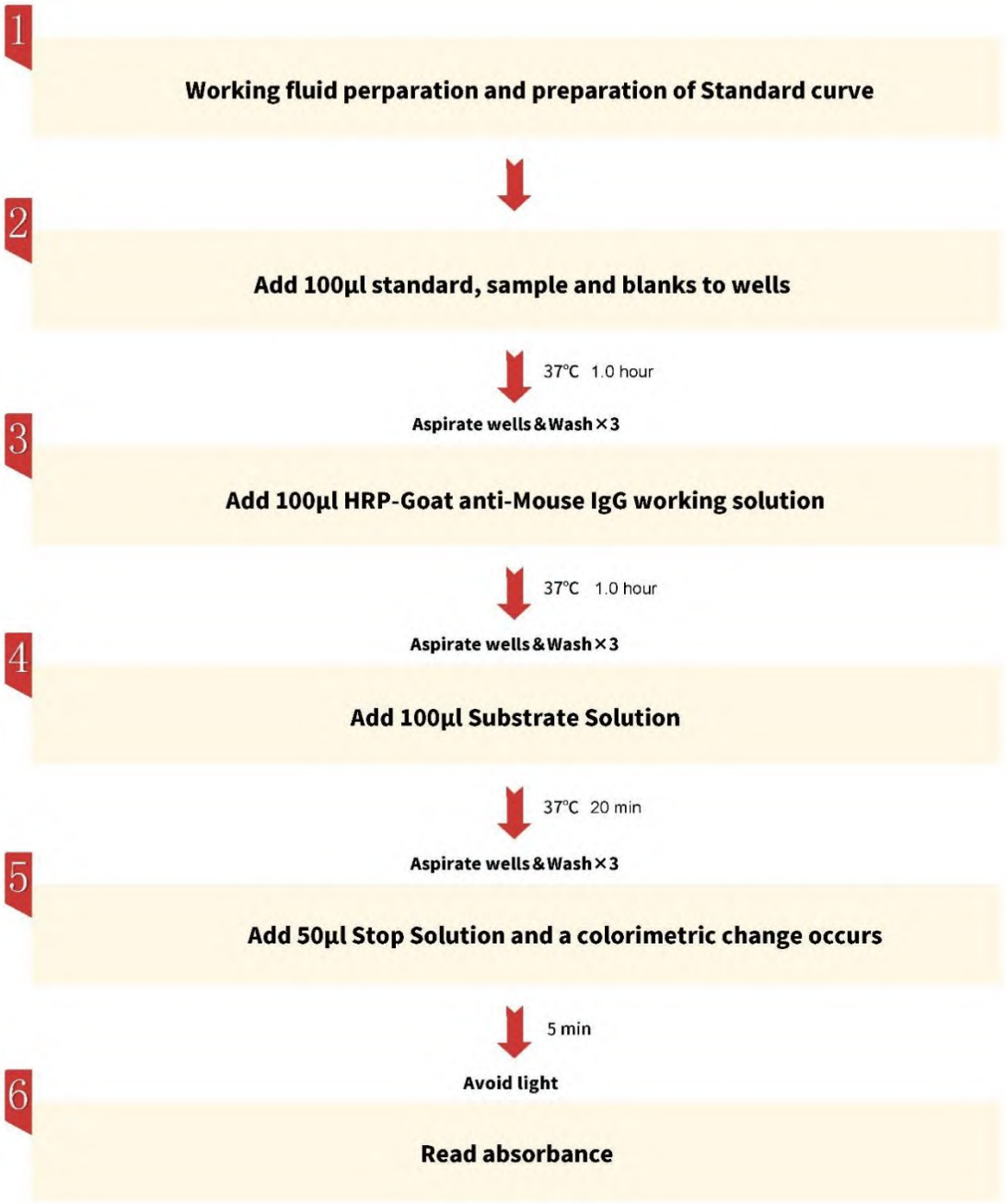
QUICK GUILD



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TYPICAL DATA

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

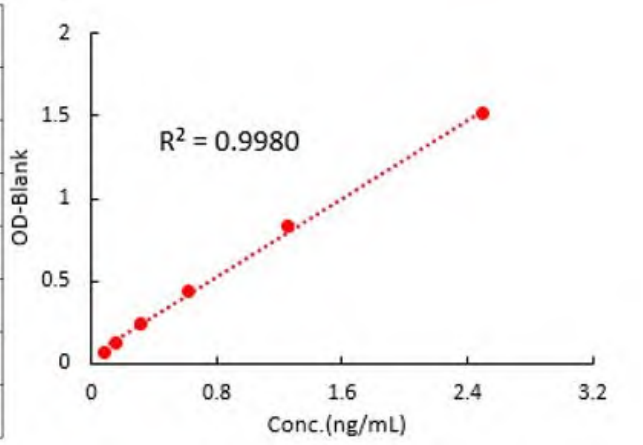
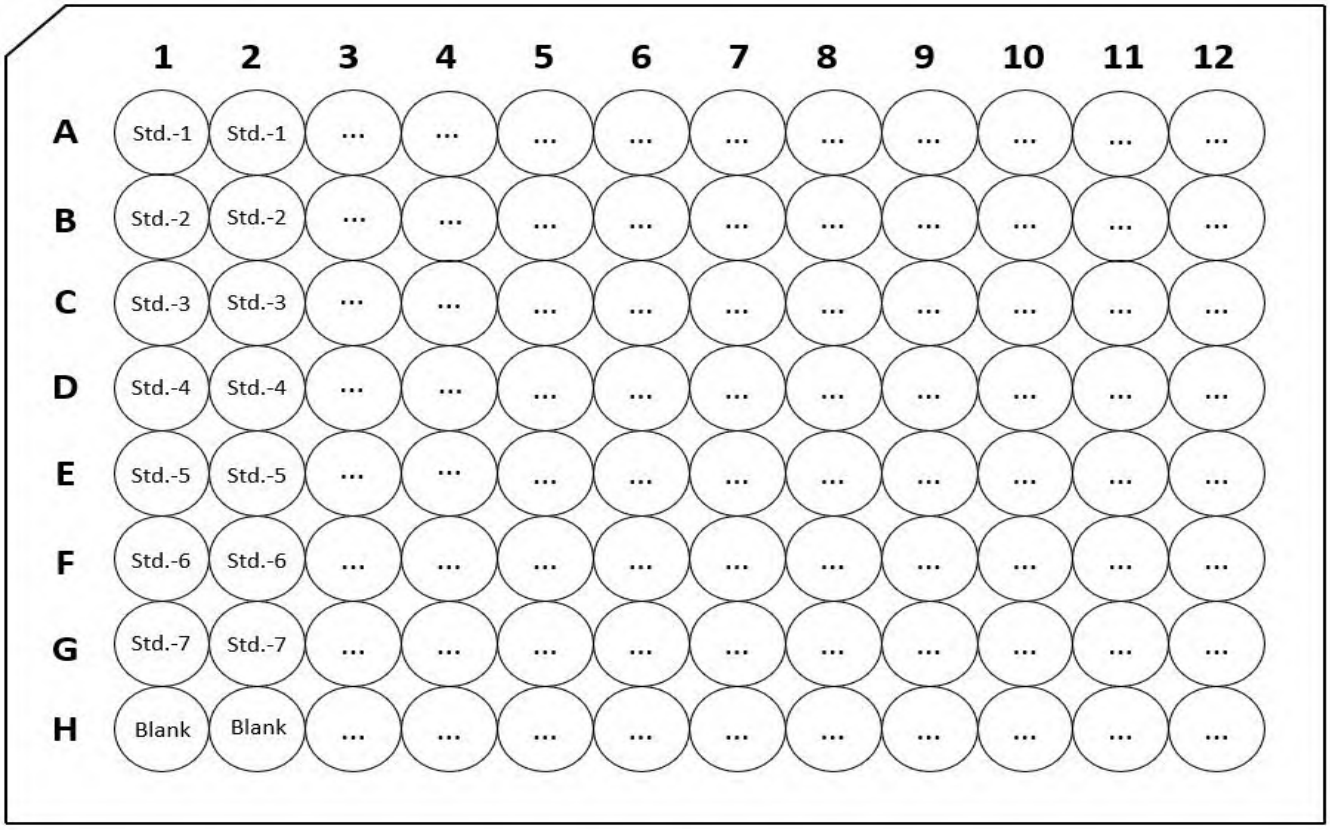


PLATE LAYOUT



Note: Blank is a Blank Dilution Buffer hole.

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TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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