P001-EN.02

Anti-CD19 (FMC63) CAR Immunogenicity ELISA Kit

Pack Size: 96 tests

Catalog Number： RAB-P001

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

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

INTENDED USE

This kit is developed for detection of FMC63 scFv Antibody in ADA assay. It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

FMC63 is an IgG2a mouse monoclonal antibody specific for CD19, which is a target for the immunotherapy of B lineage leukemias and lymphomas. FMC63 scFv is the most commonly used ectodomain component of CD19-specific CARs. So far, most of reported CART19 trials contain the anti-CD19 scFv derived from FMC63, including the two FDA-approved CARs Kymriah and Yescarta.Anti-fmc63 scFv antibody can specifically bind to the antigen recognition epitope of fmc63 scFv on anti-CD19 car, and it shows high specificity and sensitivity, it's used to detect the expression of fmc63 scFv derived car.

This assay kit is used to measure the levels of FMC63 ADA by employing a standard bridging -ELISA format. Attach the Mouse FMC63 scFv to the microplate, First add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Mouse FMC63 scFv to the plate, incubate and wash the wells. Next add Streptavidin-HRP 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 FMC63 ADA 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 FMC63 ADA bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAB001-C01	High-bind Plate	1 plate	Solid	2-8°C	2-8°C
RAB001-C02	Mouse FMC63 scFv	25 μg	Powder	2-8°C	-70°C
RAB001-C03	FMC63 ADA Standard	15 μg	Powder	2-8°C	-70°C
RAB001-C04	Biotin-Mouse FMC63 scFv	20 μg	Powder	2-8°C	-70°C
RAB001-C05	Streptavidin-HRP	50 μL	Liquid	2-8°C, avoid light	2-8°C, avoid light

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RAB001-C06	Coating Buffer	12 mL	Liquid	2-8℃	2-8℃
RAB001-C07	10xWashing Buffer	50 mL	Liquid	2-8℃	2-8℃
RAB001-C08	Blocking Buffer	50 mL	Liquid	2-8℃	2-8℃
RAB001-C09	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RAB001-C10	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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

Centrifuge;

37ºC Incubator;

10 µL, 200 µL and 1000 µL precision pipettes;

10 µL, 200 µL and 1000 µL pipette tips;

Multichannel pipettes;

Tubes;

Graduated cylinder to prepare Wash Solution;

Deionized or distilled water to dilute 10× Washing Buffer;

STORAGE

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

The opened kit should be stored per TABLE 1. The shelf life is 30 days from the date of opening.

Note: a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in a 37 ºC incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

2. 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

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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.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
RAB001-C02	Mouse FMC63 scFv	25 μg	200 μg/mL	125 μL water
RAB001-C03	FMC63 ADA Standard	15 μg	100 μg/mL	150 μL water
RAB001-C04	Biotin-Mouse FMC63 scFv	20 μg	100 μg/mL	200 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 **Dilution Buffer**:

Dilute **Blocking Buffer (RAB001-C08)** at 1:3 with 1×Washing Buffer. For example: 10 mL Blocking Buffer (RAB001-C08) add 30 mL 1×Washing Buffer.

The user should determine the dosage according to the experimental dosage, avoid cannot satisfy the requirement.

2. Coating

1) Dilute **Mouse FMC63 scFv** stock solution (200 μg/mL) to 1.0 μg/mL with **Coating Buffer** to make **Mouse FMC63 scFv** working solution.
2) Add 100 μL of **Mouse FMC63 scFv** working solution (1.0 μg/mL) to each well, seal the plate with microplate sealing film and incubate overnight (or 16 hours) at 4℃.

3. Washing

Remove the remaining solution by aspiration, add 300 μL of **1×Washing Buffer** to each well, gently tap the plate for 1 minute, 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.

4. Blocking

Add 300 μL **Blocking Buffer** to each well, seal the plate with microplate sealing film and incubate at **room**

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temperature for 2.0 hours.

5. Washing

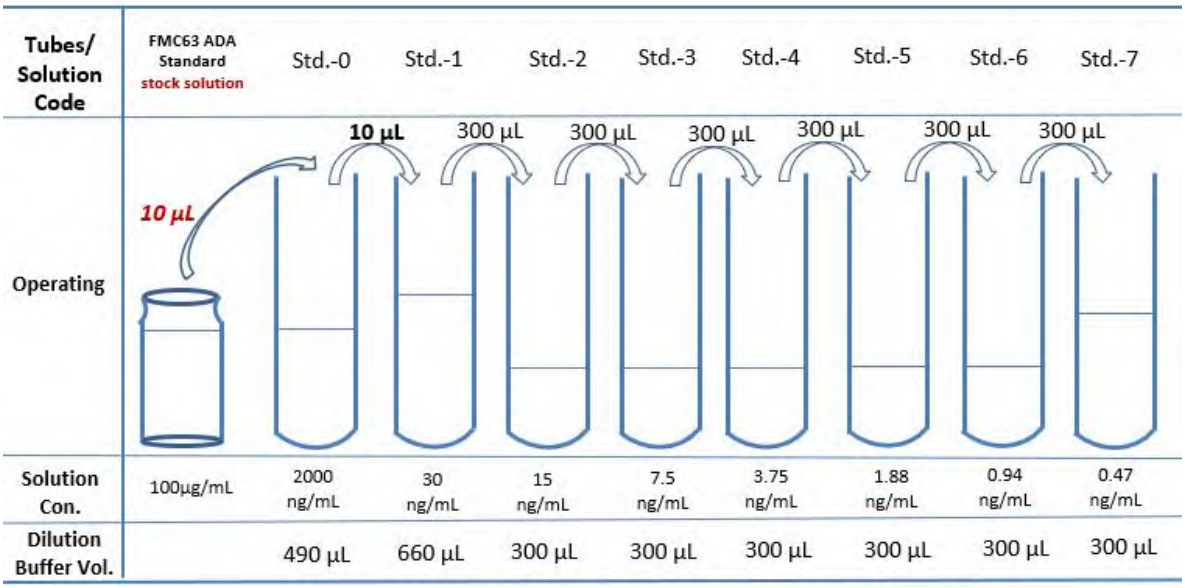
Repeat step 3.

6. Add Standard and Samples

1) Preparation of Standard curve

Make serial dilutions of the FMC63 ADA as a Standard curve with Dilution Buffer as recommended in Figure 1.

FIGURE 1. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE FMC63 ADA



2) Preparation of Samples

If the sample to be tested is the serum, dilute test sample at 1:20 with Dilution Buffer.

3) Add Samples

Add 100µL serially diluted Standard curve and samples to each well. For blank Control wells, please add 100µL Dilution Buffer. Seal the plate with microplate sealing film and incubate at **room temperature** for 1.0 hour.

7. Washing

Repeat step 3.

8. Add Biotin-Mouse FMC63 scFv

For all wells, add 100 µL **Biotin-Mouse FMC63 scFv (dilute to 0.05 µg/mL)** working solution. Seal the plate with

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microplate sealing film and incubate at **room temperature** for 1.0 hour.

9. Washing

Repeat step 3.

10. Add Streptavidin-HRP

For all wells, add 100 µL **Streptavidin-HRP (1:2000 dilute)** working solution. Seal the plate with microplate sealing film and incubate at **room temperature** for 1 hour, avoid light.

11. Washing

Repeat step 3.

12. Substrate Reaction

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

13. 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.*

14. 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: R2≥0.9900, detection range: 0.47-30 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. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

PRECAUTIONS

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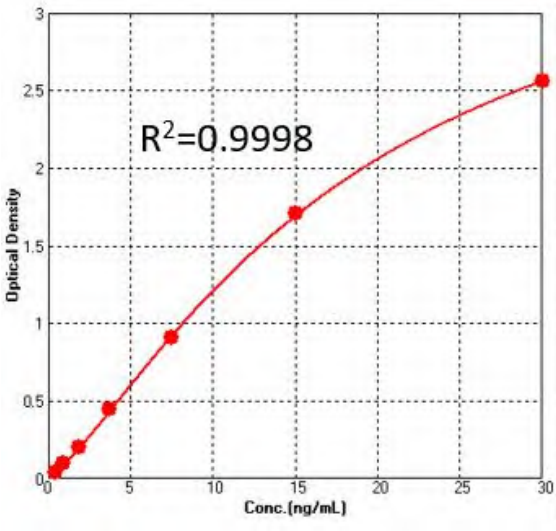
1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, warm to room temperature until the crystals have completely dissolved.
5. The kit should be stored at 2°C to 8°C.

TYPICAL DATA

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



Conc.(ng/mL)	O.D.-1	O.D.-2	Average	Corrected
30	2.672	2.522	2.597	2.560
15	1.758	1.730	1.744	1.707
7.5	0.977	0.908	0.943	0.905
3.75	0.522	0.454	0.488	0.451
1.875	0.253	0.227	0.240	0.203
0.9375	0.143	0.127	0.135	0.098
0.46875	0.081	0.070	0.076	0.038
0	0.040	0.035	0.037	0.000

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