EP152-EN.01

CD47 [Biotinylated] : SIRP alphaV2 Inhibitor Screening ELISA Kit

Pack Size: 96 tests

Catalog Number： EP-152

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 screening for inhibitors of human CD47 binding to human SIRP alphaV2.

It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

CD47 is a transmembrane protein, a cell surface glycoprotein molecule belonging to the immunoglobulin superfamily that binds to a variety of proteins, including integrins, thrombospondin 1, and signal regulatory protein-α (SIRPα). In recent years, accumulating data have demonstrated that the CD47-SIRPαV2 axis is a key immune checkpoint in different cancers, including hematological malignancies, similar to PD-1/PD-L1 in solid tumors. CD47-SIRPαV2 blockade has become a next-generation immune checkpoint blocking strategy for various malignancies after PD-1/PD-L1.

This inhibitor screening ELISA pair is designed to facilitate the identification and characterization of new CD47 pathway inhibitors. This assay employs a simple colorimetric ELISA platform, which measures the binding between immobilized human SIRP alphaV2 and in-house developed biotinylated CD47 protein. This product is uniquely suitable for rapid high-throughput screening of putative CD47 and SIRP alphaV2 inhibitors. Briefly, we provide you with a human CD47-Biotin protein, a human SIRP alphaV2 protein, an Anti-CD47 Neutralizing Antibody (as method verified Std.), and Streptavidin-HRP reagent. Your experiment will include 4 simple steps:

1) Coat the plate with human SIRP alphaV2.

2) Add your molecule of interest to the tests.

3) Add human CD47-Biotin to bind the coated human SIRP alphaV2.

4) Add Streptavidin-HRP followed by TMB or other colorimetric HRP substrate.

Finally, the ability of your compound to inhibit CD47: SIRP alphaV2 binding will be determined by comparing OD readings among different experimental groups.

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MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
EP152-C01	High-bind Plate	1 plate	Solid	2-8℃	2-8℃
EP152-C02	Human SIRP alphaV2	20 μg	Powder	2-8℃	-70℃
EP152-C03	Anti-CD47 Neutralizing Antibody	20 μg	Powder	2-8℃	-70℃
EP152-C04	Human CD47-Biotin	10 μg	Powder	2-8℃	-70℃
EP152-C05	Streptavidin-HRP	10 μg	Powder	2-8℃, avoid light	-70℃, avoid light
EP152-C06	Coating Buffer	12 mL	Liquid	2-8℃	2-8℃
EP152-C07	10xWashing Buffer	50 mL	Liquid	2-8℃	2-8℃
EP152-C08	Blocking Buffer	50 mL	Liquid	2-8℃	2-8℃
EP152-C09	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
EP152-C10	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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

Centrifuge;

37 ºC Incubator;

Single channel or multichannel pipettes with 10 μL, 200 μL and 1000 μL precision;

10 μL, 200 μL and 1000 μL pipette tips;

Test Tubes;

Graduated cylinder;

Deionized or distilled water for dilution;

STORAGE AND VALIDITY INSTRUCTIONS

The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.

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.

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REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20℃-25℃) before use.
2. Reconstitute the provided lyophilized materials to stock solutions with water as recommended in Tab.2, Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vertexing. The reconstituted stock solutions should be stored at -70℃. It is recommended not to freeze-thaw more than 2 times, the packing specification shall not be less than 5μg.

***Note**: Streptavidin-HRP stock solution should be protected from light.*

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

Catalog	Components	Amount	Stock Solution Con.	Reconstitution Buffer and Vol.
EP152-C02	Human SIRP alphaV2	20 μg	200 μg/mL	100 μL, water
EP152-C03	Anti-CD47 Neutralizing Antibody	20 μg	200 μg/mL	100 μL, water
EP152-C04	Human CD47-Biotin	10 μg	100 μg/mL	100 μL, water
EP152-C05	Streptavidin-HRP	10 μg	100 μg/mL	100 μL, water

RECOMMENDED PROTOCOL

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 (EP152-C08)** at 1:3 with **1×Washing Buffer**. For example: 10 mL **Blocking Buffer (EP152-C08)** add 30 mL **1×Washing Buffer**.

2. Coating

1) Dilute **Human SIRP alphaV2** stock solution (200 μg/mL) to 1.0 μg/mL with **Coating Buffer** to make **Human SIRP alphaV2** working solution.
2) Please leave a couple of wells uncoated for **No-Coating Control (Tab. 3)**.

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3) Add 100 µL of **Human SIRP alphaV2** 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**.

Note: *For best results, the complete removal of the* ***Human SIRP alphaV2*** *solution is essential. The use of a manifold dispenser or an auto-washer may be necessary.*

4. Blocking

Add 300 µL **Blocking Buffer** to each well, seal the plate with microplate sealing film and incubate at 37℃ for 1.5 hours.

5. Washing

Repeat step 3.

6. Add Samples

1) Make series dilution of the samples as appropriate.
2) If you intend to use the provided Anti-CD47 Neutralizing Antibody as a reference (Std.), you may dilute the antibody as recommended in Figure 1.
3) Add 50 µL of sample solution to each well according to our recommendation (Figure 2) or your own plate setup.
4) For No-Coating Control wells, please add 50 µL Dilution Buffer.

7. Binding

1) Dilute Biotinylated Human CD47 stock solution (100 µg/mL) to 0.2 µg/mL with Dilution Buffer to make Biotinylated Human CD47 working solution.
2) For No-binding ctrl. wells, please add 50 µL Dilution Buffer.
3) For all other wells, please add 50 µL Biotinylated Human CD47 working solution to the wells and mix the samples by gently tapping the plate. **Seal the plate with microplate sealing film and incubate at 37℃ for 1 hour.**

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Note: *The working solution should be prepared immediately before use and should not be stored.*

FIG.1 PREPARATION OF 1:1 SERIAL DILUTIONS OF THE Anti-CD47 Neutralizing Antibody

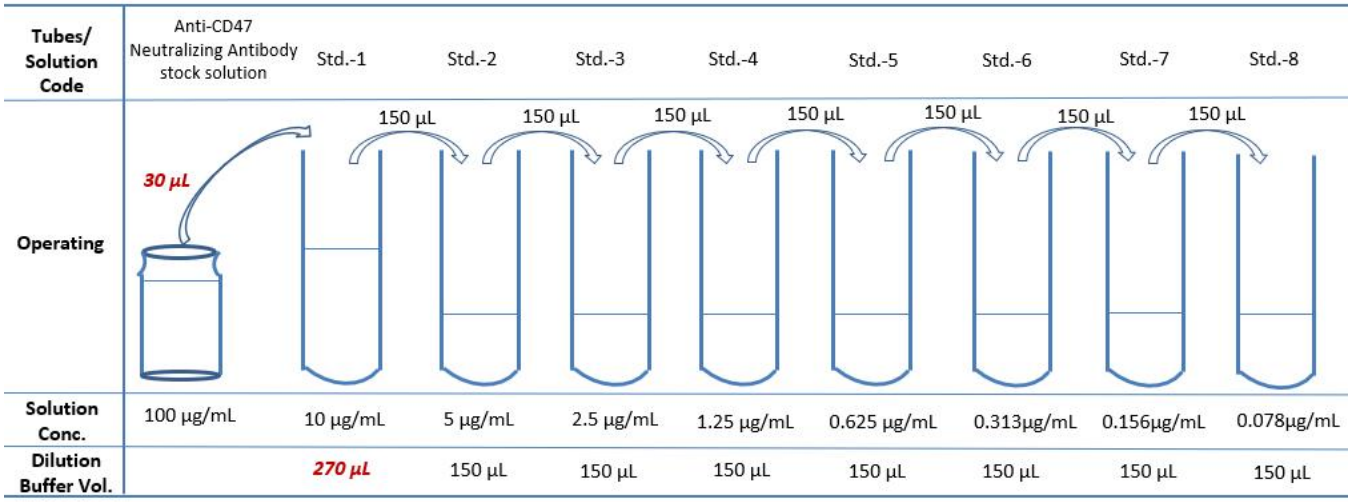
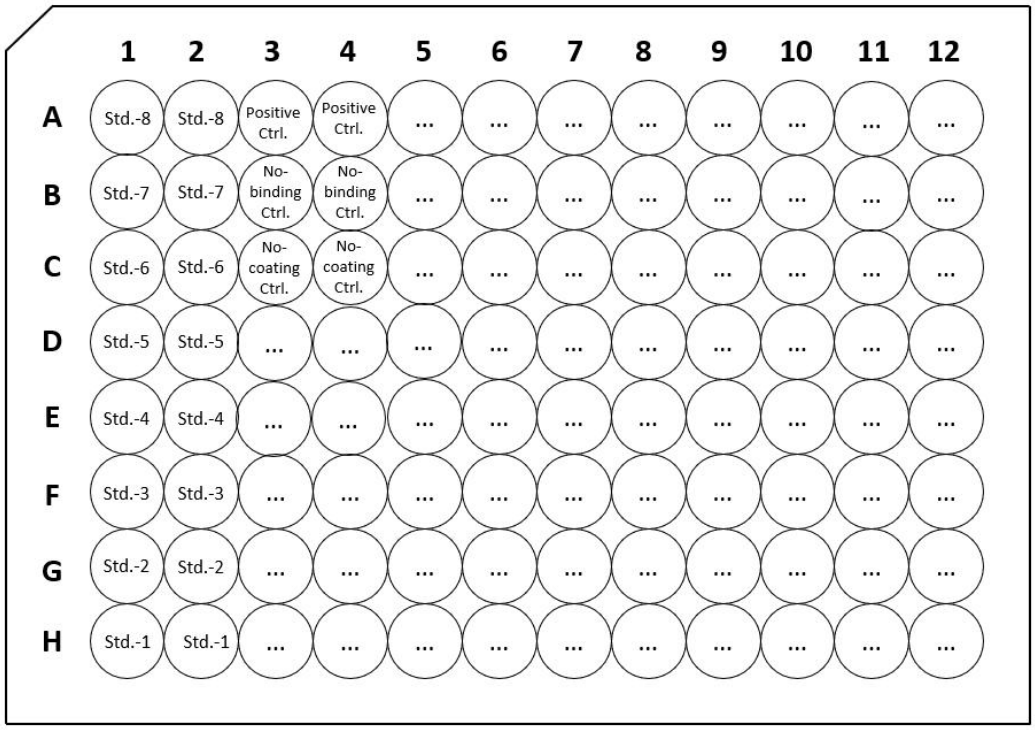


FIG.2 PLATE LAYOUT



8. Washing

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Repeat step 3.

9. Add Streptavidin-HRP

1) Dilute **Streptavidin-HRP** stock solution (100 μg/mL) to 0.05 μg/mL with **Dilution Buffer** to make **Streptavidin-HRP** working solution.
2) For all wells, add 100 μL **Streptavidin-HRP** working solution, seal the plate with microplate sealing film and incubate at 37℃ for 1 hour, **avoid light**.

Note: *The working solution should be prepared immediately before use and should not be stored.*

10. Washing

Repeat step 3.

11. Substrate Reaction

Add 100 μL **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at 37℃ for 20 minutes. Avoid light.

12. Termination

Add 50 μL **Stop Solution** to each well, and gently shake the plate to allow thorough mixing.

Note: *the color in the wells should change from blue to yellow.*

13. Data Recording

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

Note*: Subtracting the value read at* OD_{450nm} *with* OD_{630nm} *can be used to reduce the background noise.*

TAB. 3 ASSAY PROTOCOL

Steps Code	Steps	Reagents & Instruments	Reaction Conditions	Samples	No-binding Ctrl.	No-coating Ctrl.	Positive Ctrl.
1	Working fluid preparation	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
2	Coating	Human SIRP alphaV2 Working Solution	4℃ for overnight	100 μL	100 μL	—	100 μL
3	Washing	1xWashing Buffer	Wash for 3 times	300 μL	300 μL	300 μL	300 μL
4	Blocking	Blocking Buffer	37℃ for 1.5 hours	300 μL	300 μL	300 μL	300 μL
5	Washing	1xWashing Buffer	Wash for 3 times	300 μL	300 μL	300 μL	300 μL

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6	Add Samples	Samples	Incubate at 37℃ for 1.0 hour	50 µL	—	—	—
		Dilution Buffer		—	50 µL	50 µL	50 µL
7	Binding	Biotinylated Human CD47 Working Solution		50 µL	—	50 µL	50 µL
		Dilution Buffer		—	50 µL	—	—
8	Washing	1xWashing Buffer	Wash for 3 times	300 µL	300 µL	300 µL	300 µL
9	Streptavidin-HRP	Streptavidin-HRP Working Solution	37℃ for 1 hours	100 µL	100 µL	100 µL	100 µL
10	Washing	1xWashing Buffer	Wash for 3 times	300 µL	300 µL	300 µL	300 µL
11	Substrate Reaction	Substrate Solution	37℃ for 20 minutes	100 µL	100 µL	100 µL	100 µL
12	Termination	Stop Solution	Mix by gentle tapping	50 µL	50 µL	50 µL	50 µL
13	Data Recording	UV/Vis spectrophotometer	Measure absorbance at 450 nm, with the correction wavelength set at 630 nm				

Note for TAB. 3:

1) ***Samples:*** *Your samples of interest.*
2) ***No-binding Ctrl.:*** *Reaction without* ***Human CD47-Biotin*** *added. The absorbance should be around 0.05(< 0.1) at 450 nm.*
3) ***No-coating Ctrl.:*** *Reaction without* ***Human SIRP alphaV2*** *coated on the wells. The absorbance should be around 0.05(< 0.1) at 450 nm.*
4) ***Positive Ctrl.:*** *Determined the max value in 450nm absorbance, when out of inhibitors.*
5) *It is recommended that all samples, controls and standards should be done in duplicates.*

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. This kit should be used according to the provided instructions.
3. Do not mix reagents from different lots.
4. Bring all reagents and samples to room temperature (20℃-25℃) before use.
5. This kit should be stored at 2℃-8℃.
6. Please prepare the working solution of each component according to the needs of the experiment. Except for 1x Washing Buffer, all prepared working solution is for one-time use and cannot be stored.

METHOD VERIFICATION

INHIBITION OF CD47 [BIOTINYLATED]: SIRP ALPHAV2 BINDING BY ANTI-CD47 NEUTRALIZING ANTIBODY

Serial dilutions of Anti-CD47 Neutralizing Antibody (Catalog # EP152-C03) (1:1 serial dilution, from 10 µg/mL to

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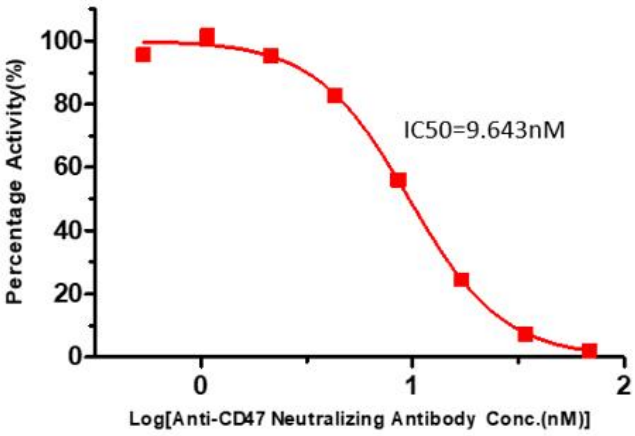
0.078 μg/mL (68.493-0.535 nM)) was added into SIRP alphaV2: CD47-Biotin binding reactions. The assay was performed according to the above-described protocol. Background was subtracted from data points prior to log transformation and curve fitting.



Anti-CD47 Neutralizing Antibody Conc.(ug/mL)	Anti-CD47 Neutralizing Antibody Conc.(nM)	Mean Abs.(OD450)	Percentage Activity(%)
0.000	0.000	3.047	100.000
0.078	0.535	2.913	95.603
0.156	1.070	3.097	101.641
0.313	2.140	2.902	95.242
0.625	4.281	2.517	82.608
1.25	8.562	1.706	55.994
2.5	17.123	0.747	24.524
5	34.247	0.225	7.394
10	68.493	0.066	2.177

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