EP165-EN.01

TNF-alpha: TNFR1[HRP] Inhibitor Screening ELISA Kit-Pre coated plate

Pack Size: 96 tests

Catalog Number: EP-165

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 TNF-alpha binding to human TNFR1
It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

This inhibitor screening ELISA kit is designed to facilitate the identification and characterization of new TNF-alpha pathway inhibitors. The assay takes advantage of our in house-developed binding of HRP human TNFR1 to immobilized human TNF-alpha in a functional ELISA assay, and employs a simple colorimetric ELISA platform. Briefly, we provide you with a human HRP TNFR1 protein, a human TNF-alpha protein, an anti-TNF-alpha neutralizing antibody (as method verified Std.), and Streptavidin-HRP reagent. Your experiment will include 3 simple steps:

a) Add your molecule of interest to the tests.

b) Add HRP-human TNFR1 to bind the coated human TNF-alpha.

c) Add TMB or other colorimetric HRP substrate.

Finally, the half maximal inhibitory concentration (IC50) of your compound to TNF-alpha: TNFR1 binding will be determined by comparing OD readings among different experimental groups.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED (pls modify according to COA)

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Catalog	Components	Size	Format	Storage	
EP165-C01	Human TNF-alpha coated Plate	1plate	Solid	2-8℃	
EP165-C02	Anti-TNF-alpha Neutralizing Antibody	10μg	Powder	2-8℃	-70℃ after reconstitution, avoid freeze-thaw cycles
EP165-C03	HRP-Human TNFR1	10μg	Powder	2-8℃, avoid light	
EP165-C04	20xWashing Buffer	50mL	Liquid	2-8℃	
EP165-C05	1X Dilution Buffer	50mL	Liquid	2-8℃	
EP165-C06	Substrate Solution	12mL	Liquid	2-8℃, avoid light	
EP165-C07	Stop Solution	7mL	Liquid	2-8℃	

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450 nm/630nm 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

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 kit should be stored as TABLE 1 after the reconstitution of lyophilized materials. The shelf life is 30 days from

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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. Restore all reagents and samples to room temperature (20-25℃) before use.

2. Reconstitute the provided lyophilized materials to stock solutions with sterile deionized water as recommended in Tab.2, Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vortex. The reconstituted stock solutions should be stored at -70℃. **Avoid freeze-thaw cycles**.

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.
EP165-C02	Anti-TNF-alpha Neutralizing Antibody	10μg	100 μg/mL	100μL, water
EP165-C03	HRP-Human TNFR1	10μg	100 μg/mL	100μL, water

RECOMMENDED PROTOCOL

1. Working solution preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 25 mL 20×Washing Buffer with ultrapure water/deionized water to 500 mL.

2. Add Samples

1)Make serial dilution of the samples as appropriate.

2)If you intend to use the provided Anti-TNF-alpha 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.

3.Binding

1) Dilute HRP-Human TNFR1 stock solution (100 μg/mL) to 0.5μg/mL with Dilution Buffer to make HRP-Human

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TNFR1 working solution.

2) For No-binding control wells, please add 50 µL Dilution Buffer.

3) For all other wells, please add 50 µL HRP-Human TNFR1 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 0.5 hour.

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-TNF-alpha Neutralizing Antibody

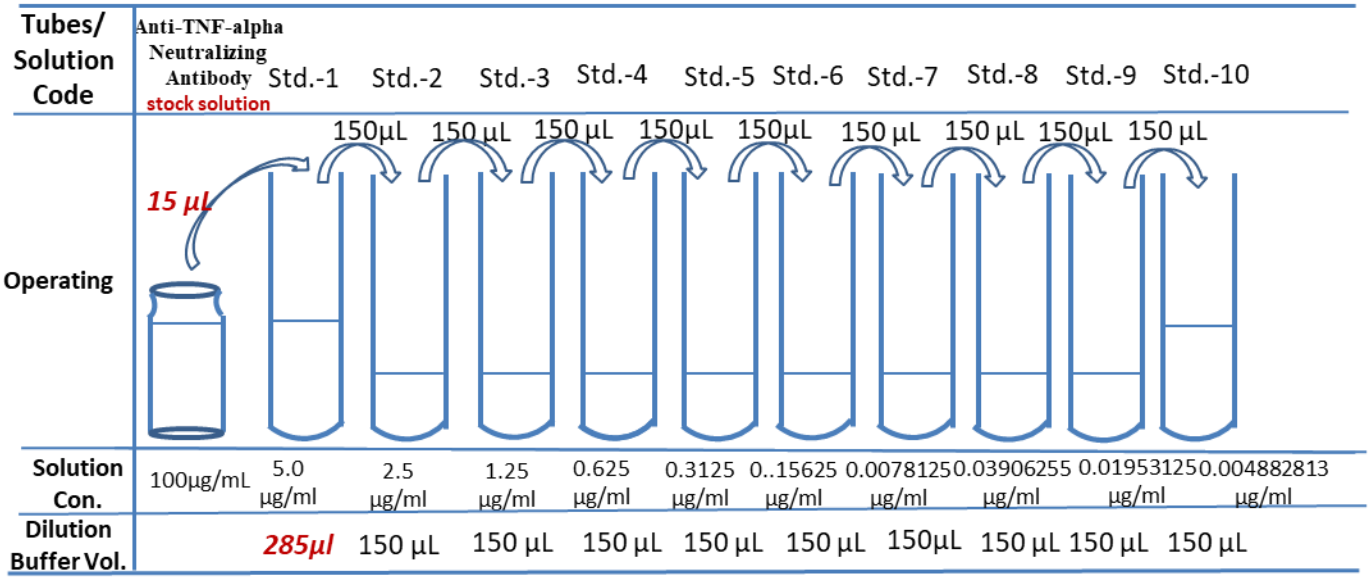
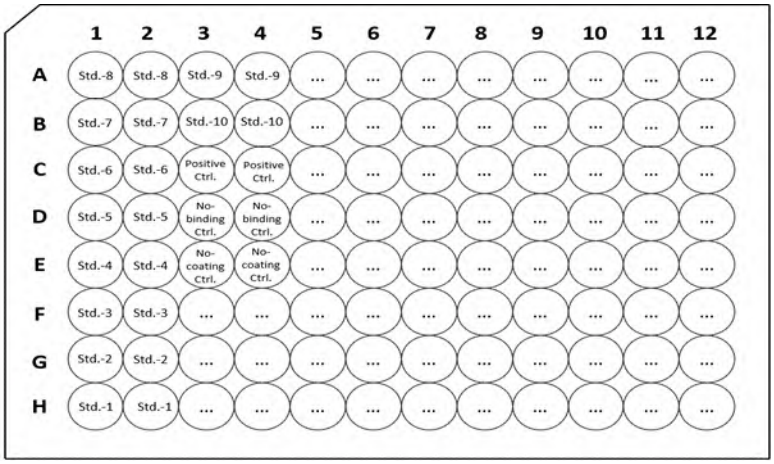


FIG.2 PLATE LAYOUT



4.Washing

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

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minute, remove any remaining 1×Washing Buffer by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the washing step above for three times

5.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.

6.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.*

7.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.*

SIMPLIFIED PROTOCOL

TABLE. 3 ASSAY PROTOCOL

Steps Code	Steps	Reagents & Instruments	Reaction Conditions	Samples	No-binding Ctrl.	No-coating Ctrl.	Positive Ctrl.
1	Add Samples	Samples	—	50 µL	—	—	—
		Dilution Buffer		—	50 µL	50 µL	50 µL
2	Binding	HRP-Human TNFR1 Working Solution	Mix by gentle tapping, incubate at 37℃ for 0.5 hours	50 µL	—	50 µL	50 µL
		Dilution Buffer		—	50 µL	—	—
3	Washing	1XWash Buffer	Wash for 3 times	300 µL	300 µL	300 µL	300 µL
4	Substrate Reaction	Substrate Solution	37℃ for 20 minutes	100 µL	100 µL	100 µL	100 µL
5	Termination	Stop Solution	Mix by gentle tapping	50 µL	50 µL	50 µL	50 µL
6	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 HRP-Human TNFR1 added. The absorbance should be around 0.05(< 0.1) at 450 nm.*
3) *Positive Ctrl.: Determined the max value in 450nm absorbance, when out of inhibitors.*

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4) *It is recommended that all samples, controls and standards should be done in duplicates.*

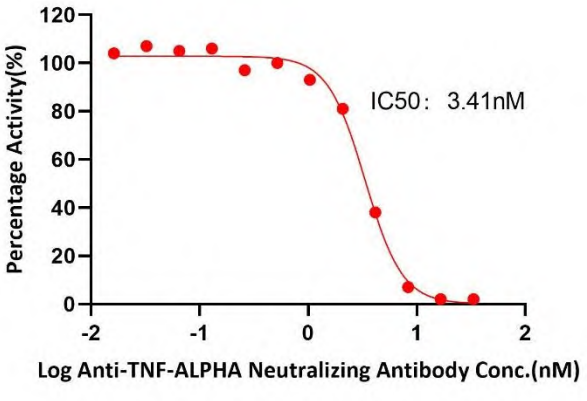
PRECAUSIONS

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. All reagents should be balanced 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 TNF-alpha: TNFR1[HRP] BINDING BY ANTI- TNF-alpha NEUTRALIZING ANTIBODY

Serial dilutions of ANTI- TNF-alpha Neutralizing Antibody (Catalog # EP165-C02) (1:1 serial dilution, from 5μg/mL to 0.002441406μg/mL) was added into TNF-alpha: TNFR1[HRP] binding reactions. The assay was performed according to the protocol described below. Background was subtracted from data points prior to log transformation and curve fitting (QC tested).



Anti-TNF-alpha Neutralizing Antibody conc(ug/mL)	Anti-TNF-alpha Neutralizing Antibody Conc(nM)	MeanAbs.(OD 450)	Percentage Activity(%)
0	0	2.856	100%
0.002	0.016	2.973	104%
0.005	0.033	3.052	107%
0.010	0.065	2.986	105%
0.020	0.130	3.025	106%
0.039	0.260	2.763	97%
0.078	0.521	2.855	100%
0.156	1.042	2.663	93%
0.313	2.083	2.306	81%
0.625	4.167	1.086	38%
1.250	8.333	0.212	7%
2.500	16.667	0.069	2%
5.000	33.333	0.060	2%
No Binding		0.051	

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For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The example data is for reference only.

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