EP168-EN.01

TL1A [Biotinylated] : DR3 Inhibitor Screening ELISA Kit

Pack Size: 96 tests

Catalog Number： EP-168

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 designed for screening of inhibitors of human DR3 binding to human TL1A.

It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

TNF-like cytokine 1A (TL1A) and its receptors, death receptor 3 (DR3) and decoy receptor 3 (DcR3) are members of the TNF and TNF receptor superfamilies of proteins, respectively. Binding of APC-derived TL1A to lymphocytic DR3 provides co-stimulatory signals for activated lymphocytes. DR3 signaling affects not only the proliferative activity of and cytokine production by effector lymphocytes, but also critically influences the development and suppressive function of regulatory T-cells. Whereas, DcR3 restricts the function of the TL1A/DR3 complex: attenuating T-cell activation and downregulating the secretion of pro-inflammatory cytokines. Together with DR3 and DcR3, TL1A constitutes a cytokine system that actively interferes with the regulation of immune responses.

This inhibitor screening ELISA kit is designed to facilitate the identification and characterization of new Human DR3 pathway inhibitors. The assay takes advantage of our in house-developed binding of biotinylated Human TL1A to immobilized Human DR3 in a functional ELISA assay and employs a simple colorimetric ELISA platform. Briefly, we provide you with a Human DR3 protein, a Biotinylated Human TL1A, an anti- TL1A neutralizing antibody (as method verified Std.), and Streptavidin-HRP reagent. Your experiment will include 4 simple steps:

1) Coat the plate with Human DR3.

2) Add your molecule of interest to the tests.

3) Add Human TL1A-Biotin to bind the coated Human DR3.

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

Finally, the ability of your compound to inhibit DR3: TL1A binding will be determined by comparing OD readings among different experimental groups.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size	Format	Storage

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		(96 tests)		Unopened	Opened
EP168-C01	High-bind Plate	1 plate	Solid	2-8℃	2-8℃
EP168-C02	Human DR3	100 μg	Powder	2-8℃	-70℃
EP168-C03	Biotinylated Human TL1A	6 μg	Powder	2-8℃	-70℃
EP168-C04	Anti-Human TL1A Neutralizing Antibody	100 μg	Powder	2-8℃	-70℃
EP168-C05	Streptavidin-HRP	5 μg	Powder	2-8℃, avoid light	-70℃, avoid light
EP168-C06	Coating Buffer	12 mL	Liquid	2-8℃	2-8℃
EP168-C07	20×Washing Buffer	50 mL	Liquid	2-8℃	2-8℃
EP168-C08	Blocking Buffer	50 mL	Liquid	2-8℃	2-8℃
EP168-C09	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
EP168-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.*

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b. Find the expiration date on the outside packaging.

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 5 μg.

***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.
EP168-C02	Human DR3	100 μg	200 μg/mL	500 μL, water
EP168-C03	Biotinylated Human TL1A	6 μg	60 μg/mL	100 μL, water
EP168-C04	Anti-Human TL1A Neutralizing	100 μg	200 μg/mL	500 μL, water
EP168-C05	Streptavidin-HRP	5 μg	50 μg/mL	100 μL, water

RECOMMENDED PROTOCOL

1. Working fluid preparation

1.1 Preparation of **1×Washing Buffer**:

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

1.2 Preparation of **Dilution Buffer**:

Dilute **Blocking Buffer (EP168-C08)** at 1:3 with **1×Washing Buffer**. For example: 10 mL **Blocking Buffer (EP168-C08)** add 30 mL **1×Washing Buffer**.

2. Coating

1) Dilute **Human DR3** stock solution (200 μg/mL) to 10 μg/mL with **Coating Buffer** to make **Human DR3** working solution.

2) Please leave a couple of wells uncoated for **No-Coating Control (Tab. 3)**.

3) Add 100 μL of **Human DR3** working solution (10 μg/mL) to each well, seal the plate with microplate sealing film

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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 DR3*** *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-TL1A 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 TL1A stock solution (60 µg/mL) to 0.4 µg/mL with Dilution Buffer to make Biotinylated Human TL1A 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 TL1A 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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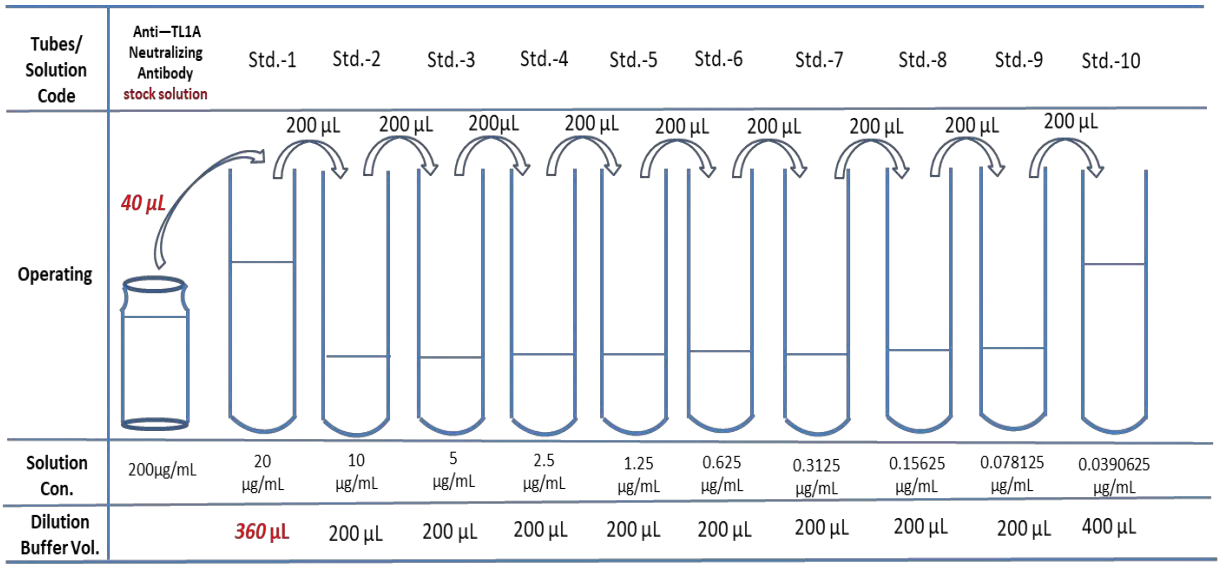
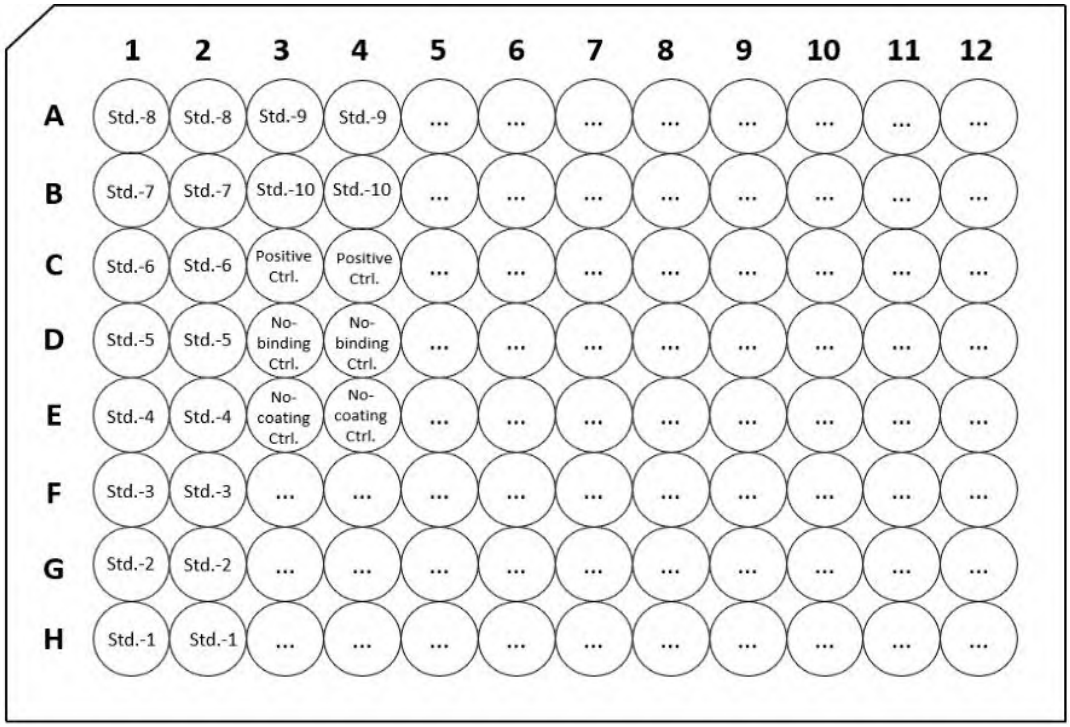
FIG.1 PREPARATION OF 1:1 SERIAL DILUTIONS OF THE Anti-TL1A Neutralizing Antibody



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

FIG.2 PLATE LAYOUT



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8. Washing

Repeat step 3.

9. Add Streptavidin-HRP

1) Dilute **Streptavidin-HRP** stock solution (50 μg/mL) to 0.1 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* $OD_{450\,nm}$ *with* $OD_{630\,nm}$ *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 DR3 Working	4℃ for overnight	100 μL	100 μL	—	100 μL

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		Solution					
3	Washing	1×Washing 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	1×Washing Buffer	Wash for 3 times	300 µL	300 µL	300 µL	300 µL
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 TL1A Working Solution		50 µL	—	50 µL	50 µL
		Dilution Buffer		—	50 µL	—	—
8	Washing	1×Washing 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	1×Washing 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 TL1A-Biotin*** *added. The absorbance should be around 0.05(< 0.1) at 450 nm.*

3) ***No-coating Ctrl.:*** *Reaction without* ***Human DR3*** *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

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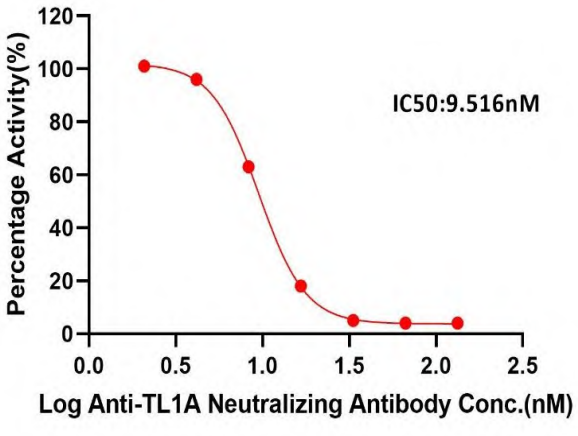
1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. This kit should be used according to the provided instructions.
3. Do not mix reagents from different lots.
4. Bring all reagents and samples to room temperature (20℃-25℃) before use.
5. This kit should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

METHOD VERIFICATION

INHIBITION OF TL1A [BIOTINYLATED]: DR3 BINDING BY ANTI-CD47 NEUTRALIZING ANTIBODY

Serial dilutions of Human Anti-TL1A Neutralizing antibody (Catalog # EP168-C04) (1:1 serial dilution, from 20 μg/mL to 0.3125 μg/mL) was added into Human DR3: Biotinylated Human TL1A 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-TL1A Neutralizing Antibody(μg/ml)	Anti-TL1A Neutralizing Antibody.(nM)	Mean Abs.(OD450)	Percentage Activity(%)
0.000	0.000	2.334	100%
0.313	2.083	2.350	101%
0.625	4.167	2.234	96%
1.250	8.333	1.470	63%
2.500	16.667	0.419	18%
5.0	33.333	0.127	5%
10	66.667	0.093	4%
20	133.333	0.082	4%

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