EP132-EN.03

IL-4 [Biotinylated]: IL-4Rα Inhibitor Screening ELISA Kit

Pack Size: 96 tests

Catalog Number： EP-132

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

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

INTENDED USE

The kit is useful for screening for inhibitors of human IL-4 binding to human IL-4 R alpha.

It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

Interleukin-4, is a cytokine that induces differentiation of naive helper T cells (Th0 cells to Th2 cells). In the presence of IL-4 and IL-13, cytokines that are produced in a Th-2 type response, particularly during allergy and parasitic infections, macrophages become differentially activated, and this cytokine is a ligand for interleukin 4 receptor. The interleukin 4 receptor also binds to IL13, which may contribute to many overlapping functions of this cytokine and IL13. STAT6, a signal transducer and activator of transcription, has been shown to play a central role in mediating the immune regulatory signal of this cytokine.

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

1) Coat the plate with human IL-4 R alpha.

2) Add your molecule of interest to the tests.

3) Add human IL-4-Biotin to bind the coated human IL-4 R alpha.

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

Finally, the ability of your compound to inhibit IL-4: IL-4 R alpha 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
EP132-C01	High-bind Plate	1 plate	Solid	2-8℃	2-8℃
EP132-C02	Human IL-4 R alpha	30 μg	Powder	2-8℃	-70℃
EP132-C03	Human IL-4	20 μg	Powder	2-8℃	-70℃
EP132-C04	Human IL-4-Biotin	10 μg	Powder	2-8℃	-70℃
EP132-C05	Streptavidin-HRP	10 μg	Powder	2-8℃, avoid light	-70℃, avoid light
EP132-C06	Coating Buffer	12 mL	Liquid	2-8℃	2-8℃
EP132-C07	10xWashing Buffer	50 mL	Liquid	2-8℃	2-8℃
EP132-C08	Blocking Buffer	50 mL	Liquid	2-8℃	2-8℃
EP132-C09	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
EP132-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/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 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℃. **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.
EP132-C02	Human IL-4 R alpha	30 μg	200 μg/mL	150 μL, water
EP132-C03	Human IL-4	20 μg	100 μg/mL	200 μL, water
EP132-C04	Human IL-4-Biotin	10 μg	100 μg/mL	100 μL, water
EP132-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 (EP132-C08)** at 1:3 with **1×Washing Buffer**. For example: 10 mL **Blocking Buffer (EP132-C08)** add 30 mL **1×Washing Buffer**.

2. Coating

1) Dilute **Human IL-4 R alpha** stock solution (200 μg/mL) to 2 μg/mL with **Coating Buffer** to make **Human IL-4 R alpha** working solution.
2) Please leave a couple of wells uncoated for **No-Coating Control (Tab. 3)**.
3) Add 100 μL of **Human IL-4 R alpha** working solution (2 μg/mL) to each well, seal the plate with microplate sealing film and incubate overnight (or 15 hours) at 4℃.

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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 IL-4 R alpha*** *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. At meantime, you can start to prepare your samples.

6. Add Samples

1) Make series dilution of the samples as appropriate.
2) If you intend to use the provided Human IL-4 as a reference (Std.), you may dilute the Human IL-4 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 IL-4 stock solution (100 µg/mL) to 0.02 µg/mL with Dilution Buffer to make Biotinylated Human IL-4 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 IL-4 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.

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

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FIG.1 PREPARATION OF 1:1 SERIAL DILUTIONS OF THE Human IL-4

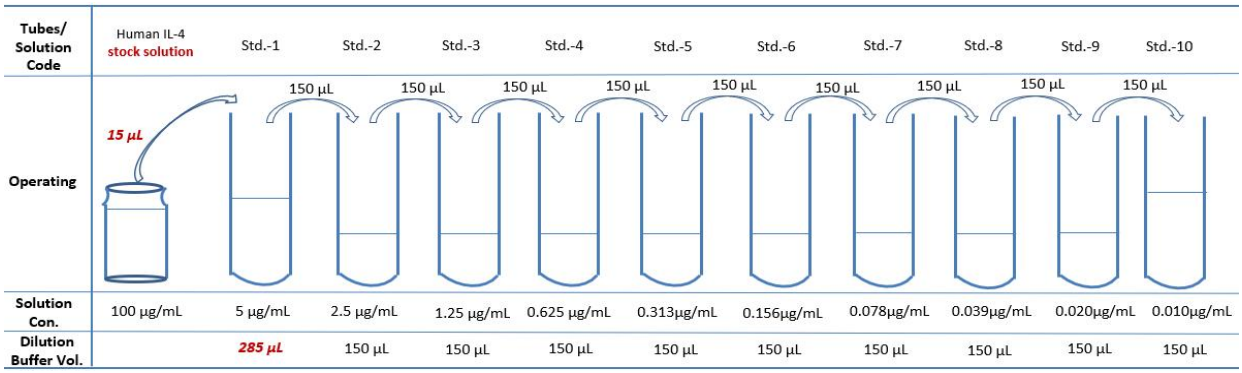
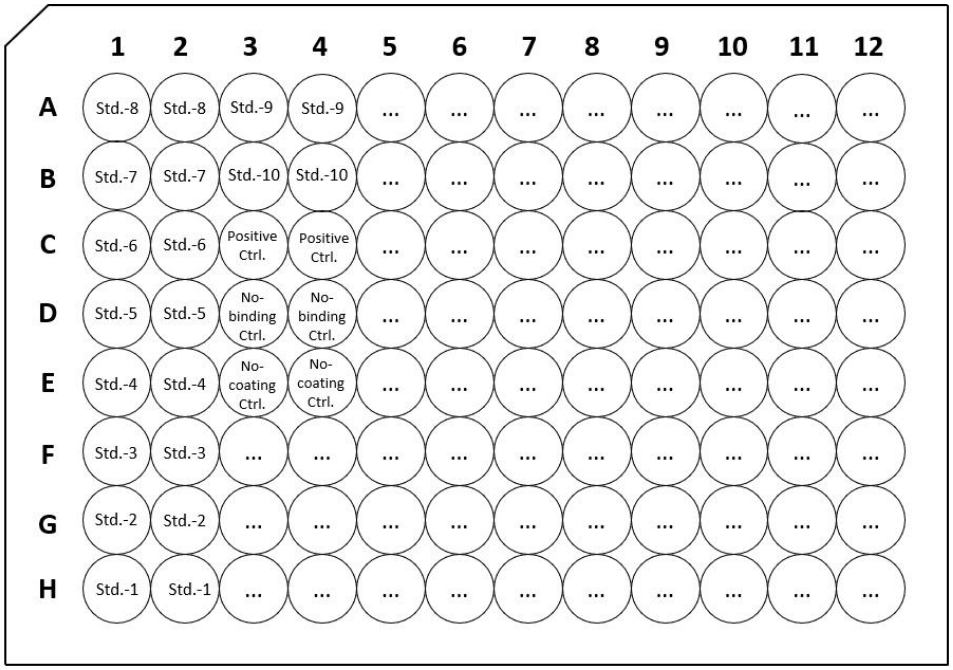


FIG.2 PLATE LAYOUT



8. Washing

Repeat step 3.

9. Add Streptavidin-HRP

1) Dilute **Streptavidin-HRP** stock solution (100 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**.

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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 IL-4 R alpha 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
6	Add Samples	Samples	Incubate at 37℃ for 1 hour	50 µL	—	—	—
		Dilution Buffer		—	50 µL	50 µL	50 µL
7	Binding	Biotinylated Human IL-4 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

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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 IL-4-Biotin*** *added. The absorbance should be around 0.05(< 0.1) at 450 nm.*
3) ***No-coating Ctrl.:*** *Reaction without* ***Human IL-4 R alpha co****ated 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.*

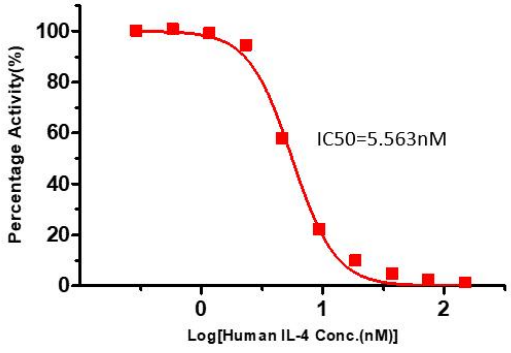
PRECAUTIONS

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 IL-4 [BIOTINYLATED]: IL-4 R ALPHA BINDING BY Human IL-4

Serial dilutions of Human IL-4(Catalog # EP132-C03) (1:1 serial dilution, from 5 μg/mL to 0.01 μg/mL (295.86-0.58 nM)) was added into IL-4 R alpha : IL-4-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.



Human IL-4 Conc.(ug/mL)	Human IL-4 Conc.(nM)	Mean Abs.(OD450)	Percentage Activity(%)
0	0.000	3.148	100.000
0.005	0.289	3.156	100.254
0.010	0.578	3.176	100.890
0.020	1.156	3.121	99.142
0.039	2.311	2.978	94.599
0.078	4.623	1.824	57.935
0.156	9.246	0.700	22.224
0.313	18.491	0.314	9.960
0.625	36.982	0.152	4.813
1.250	73.964	0.082	2.589
2.500	147.929	0.046	1.446

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