EP169-EN.01

Fc epsilon RI alpha [Biotinylated] : IgE Fc Inhibitor Screening ELISA Kit

Pack Size: 96 tests

Catalog Number： EP-169

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 IgE Fc binding to Human Fc epsilon RI alpha.

It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

High affinity immunoglobulin epsilon receptor subunit alpha (FCER1A) is also known as Fc-epsilon RI-alpha (FcERI), IgE Fc receptor subunit alpha, FCE1A. FCER1A contains two Ig-like (immunoglobulin-like) domains. FCER1A binds to the Fc region of immunoglobulins epsilon and is a high affinity receptor. FCER1A is responsible for initiating the allergic response, which binding of allergen to receptor-bound IgE leads to cell activation and the release of mediators (such as histamine) responsible for the manifestations of allergy. The same receptor also induces the secretion of important lymphokines. FCER1A plays a central role in allergic disease, coupling allergen and mast cell to initiate the inflammatory and immediate hypersensitivity responses that are characteristic of disorders such as hay fever and asthma. Briefly, we provide you with a Human IgE Fc protein, a Human Fc epsilon RI alpha-SABC, an Anti-IgE (Omalizumab) (as method verified Std.).Your experiment will include 4 simple steps:

1) Coat the plate with Human IgE Fc protein.

2) Add your molecule of interest to the tests.

3) Add Human Fc epsilon RI alpha-SABC to bind the coated Human IgE Fc protein.

4) Add TMB or other colorimetric HRP substrate.

Finally, the ability of your compound to inhibit IgE Fc : Fc epsilon RI alpha binding will be determined by comparing OD readings among different experimental groups.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
EP169-C01	High-bind Plate	1 plate	Solid	2-8°C	2-8°C
EP169-C02	Human IgE Fc	60 μg	Powder	2-8°C	-70°C
EP169-C03	Human Fc epsilon RI alpha-SABC	0.583μg	Powder	2-8°C, avoid light	-70°C, avoid light

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EP169-C04	Anti-IgE (Omalizumab)	30 μg	Powder	2-8℃	-70℃
EP169-C05	Coating Buffer	12 mL	Liquid	2-8℃	2-8℃
EP169-C06	20×Washing Buffer	50 mL	Liquid	2-8℃	2-8℃
EP169-C07	Blocking Buffer	50 mL	Liquid	2-8℃	2-8℃
EP169-C08	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
EP169-C09	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

1. Unopened kit should be stored at 2℃-8℃ upon receiving.
2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.
3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

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:Human Fc epsilon RI alpha-SABC *stock solution should be protected from light.*

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TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

Catalog	Components	Amount	Stock Solution Con.	Reconstitution Buffer and Vol.
EP169-C02	Human IgE Fc	60 μg	200 μg/mL	300 μL, water
EP169-C03	Human Fc epsilon RI alpha-SABC	0.583μg	2.12 μg/mL	275 μL, water
EP169-C04	Anti-IgE (Omalizumab)	30 μg	150 μg/mL	200 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 (EP169-C07)** at 1:3 with **1×Washing Buffer**. For example: 10 mL **Blocking Buffer (EP169-C07)** add 30 mL **1×Washing Buffer**.

2. Coating

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

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

3) Add 100 μL of **Human IgE Fc** working solution (1 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 IgE Fc*** *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.

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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-IgE (Omalizumab) 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 Human Fc epsilon RI alpha-SABC stock solution (2.12 µg/mL) to 0.053 µg/mL with Dilution Buffer to make Human Fc epsilon RI alpha-SABC working solution.

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

3) For all other wells, please add 50 µL Human Human Fc epsilon RI alpha-SABC 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°C for 1 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-IgE (Omalizumab)

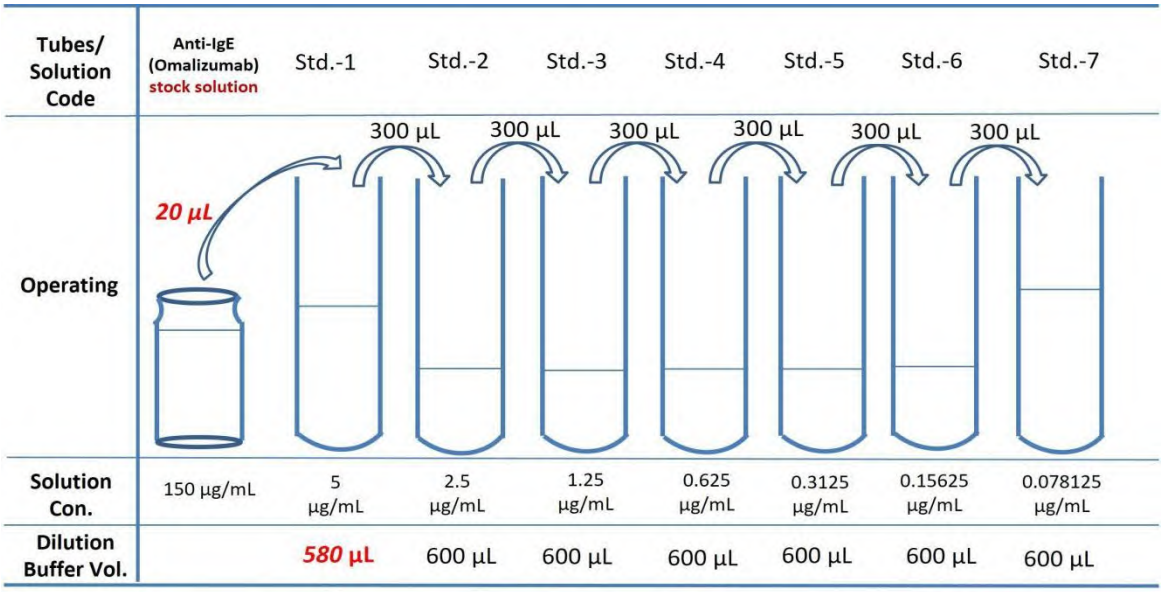

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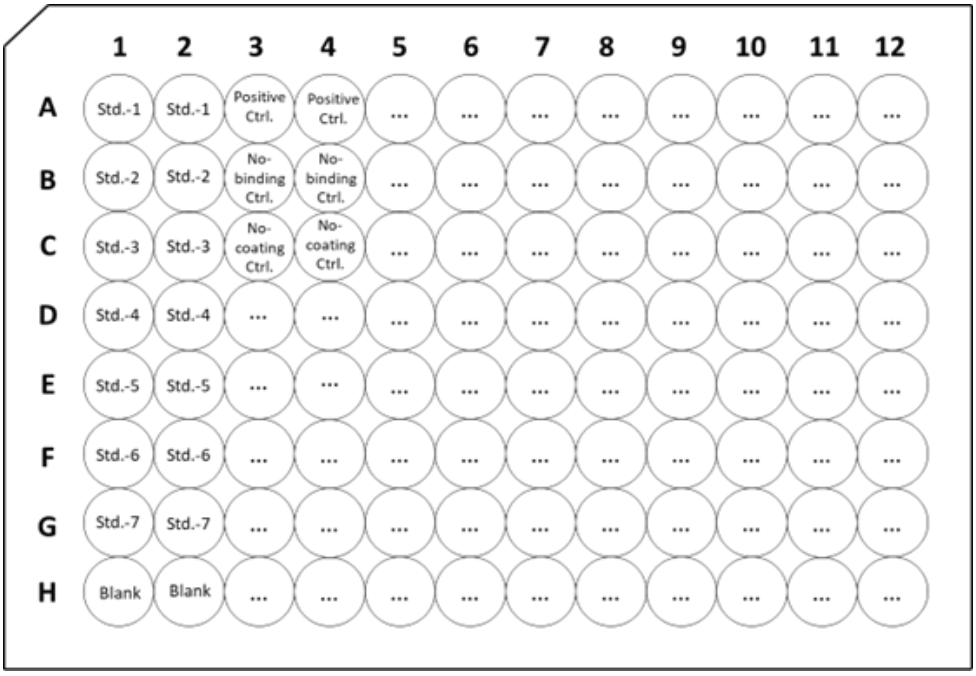
FIG.2 PLATE LAYOUT



8. Washing

Repeat step 3.

9. 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.

10. 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.*

11. 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.*

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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 IgE Fc 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	Human Fc epsilon RI alpha-SABC Working Solution	Incubate at 37℃ for 1.0 hour	50 µL	—	—	—
		Dilution Buffer		—	50 µL	50 µL	50 µL
7	Binding	Human Fc epsilon RI alpha-SABC 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	Substrate Reaction	Substrate Solution	37℃ for 20 minutes	100 µL	100 µL	100 µL	100 µL
10	Termination	Stop Solution	Mix by gentle tapping	50 µL	50 µL	50 µL	50 µL
11	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 Fc epsilon RI alpha-SABC*** *added. The absorbance should be around 0.05(< 0.1) at 450 nm.*

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

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

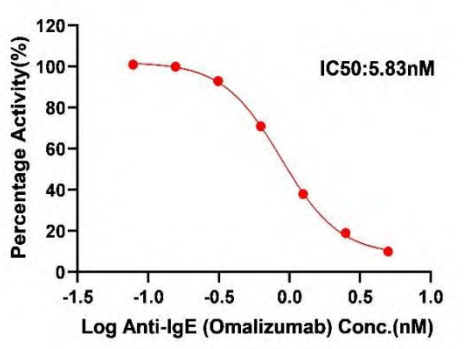
5. This kit should be stored at 2℃-8℃.

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 Fc epsilon RI alpha [BIOTINYLATED] : IgE Fc BINDING BY Anti-IgE (Omalizumab)

Serial dilutions of Anti-IgE (Omalizumab) (Catalog # EP169-C04) (1:1 serial dilution, from 5 μg/mL to 0.078125 μg/mL) was added into Human IgE Fc: Biotinylated Human Fc epsilon RI alpha 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-IgE (Omalizumab)(μg/ml)	Anti-IgE (Omalizumab)(nM)	Mean Abs.(OD450)	Percentage Activity(%)
0.000	0.000	3.344	100%
0.078	0.521	3.377	101%
0.156	1.042	3.341	100%
0.313	2.083	3.126	93%
0.625	4.167	2.386	71%
1.250	8.333	1.269	38%
2.500	16.667	0.632	19%
5.000	33.333	0.329	10%

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