FcεRI: IgE [Biotinylated] Inhibitor Screening Chemiluminescent Assay Kit

#84219
96 reactions

Description

The FcεRI: IgE [Biotinylated] Inhibitor Screening Chemiluminescent Assay Kit is an ELISA designed to measure the binding between Human FcεRI (Fc epsilon RI alpha) and IgE (immunoglobulin E). This assay kit comes with enough purified FcεRI (amino acids 26-205) and biotinylated IgE (amino acids 106-427), streptavidin-HRP, assay buffer, blocking buffer, and detection reagent for 100 enzyme reactions.

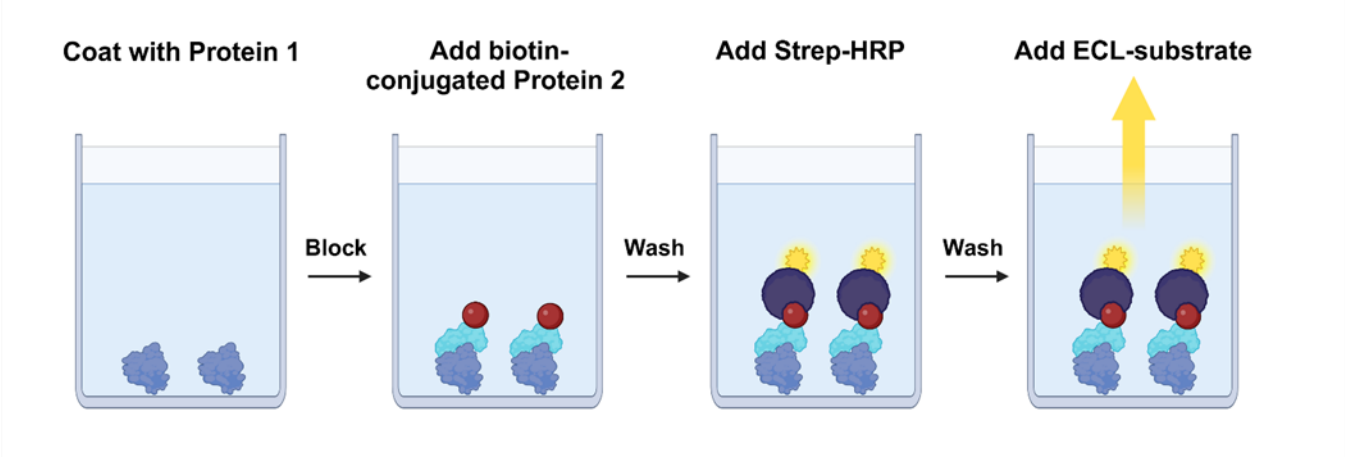

Figure 1: FcεRI: IgE [Biotinylated] Inhibitor Screening Chemiluminescent Assay Kit schematic.
A 96-well plate is coated with the FcεRI protein. After coating and blocking, biotinylated IgE is added in an optimized assay buffer. Next, unbound biotinylated IgE is washed away, and the plate is incubated with streptavidin-HRP. After a final wash, ELISA ECL substrate is added to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to IgE binding to FcεRI.

Background

IgE (immunoglobulin E) is an immunoglobulin isotype that is found only in mammals. It can be found at low levels in the blood of normal individuals and plays a role in immune responses to parasites, toxins, cancer and hypersensitivity reactions to allergens. It is linked to allergic asthma, sinusitis, allergic rhinitis, atopic dermatitis (AD), and can trigger anaphylaxis. IgE can bind to the high affinity IgE receptor (FcεRI) or the low affinity FcεRII (also known as CD23). In the presence of an allergen, IgE binds to FcεRI present in mast cells and basophils, triggering the release of histamine, leukotrienes and interleukins. CD23 has a broader expression pattern, including macrophages and B cells, and plays a role in the regulation of IgE synthesis, serum clearance and antigen presentation. IgE is highly glycosylated, and the glycosylation of N394 in human IgE is crucial for receptor binding. IgE is found at high levels in patients with SLE (systemic lupus erythematosus), RA (reumathoid arthritis) and psoriasis. The development of therapeutic antibodies targeting IgE, such as omalizumab, may be useful for the treatment of IgE- mediated inflammation and allergic diseases.

Applications

Screen or titrate compounds that inhibit the binding of FcεRI to IgE for drug discovery in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
84214-KC10^{+}	Human FcεRI, His-Tag*	10 µg	-80°C
84215-KC500^{+}	Human IgE, His-Tag, Biotin-Labeled*	500 ng	-80°C
82620-KC4	5x PP-02 Buffer	4 ml	-20°C
79743-KC25	Blocking Buffer 3	25 ml	+4°C
79742	Streptavidin-HRP	10 µl	+4°C
79670-KC6	ELISA ECL Substrate A	6 ml	Room Temp
	ELISA ECL Substrate B	6 ml	Room Temp
79699	White 96-well microplate	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- PBS Buffer (1x PBS)
- PBST Buffer (1x PBS with 0.05% Tween-20)
- Microplate reader capable of reading luminescence
- Adjustable micropipettor and sterile tips
- Orbital shaker

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

This kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control”, and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Omalizumab (Anti-IgE Antibody) (#83596) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1: Coat 96-well plate
Coat the plate one day prior to running your samples.

1. Thaw **FcεRI** on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute **FcεRI** protein to 2 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of **diluted FcεRI** to every well, except "Blank" wells.
4. Add 50 µl of **PBS** to the "Blank" wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times using 200 µl of **PBST Buffer** per well.
7. Tap the plate onto clean paper towels to remove the liquid.
8. Block the wells by adding 200 µl of **Blocking Buffer 3** to every well.
9. Incubate at Room Temperature (RT) for at least 90 minutes.
10. Wash the plate three times using 200 µl of **PBST Buffer** per well.
11. Tap the plate onto clean paper towels to remove the liquid.

Step 2: Binding reaction

1. Prepare **1x Assay Buffer** by diluting **5x PP-02 Assay Buffer** 5-fold with distilled water.
2. Add 20 µl of **1x Assay Buffer** to every well.
3. Prepare the **Test Inhibitor/Blocker** (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   3.1 If the Test Inhibitor/Blocker is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration using 1x Assay Buffer.

   For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   OR

   3.2 If the Test Inhibitor/Blocker is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with 1x

Assay Buffer (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in 1x Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

4. Add 5 µl of **Test Inhibitor** to each well labeled as "Test Inhibitor".
5. Add 5 µl of **Diluent Solution** to the "Positive Control" and "Blank" wells.
6. Preincubate at RT for 30 minutes with gentle agitation.

   Note: If testing inhibitors/blockers that target IgE, we recommend preincubating them with IgE.
7. Thaw **IgE-Biotin** on ice. Briefly spin the tube containing the protein to recover its full content.
8. Dilute **IgE-Biotin** to 0.2 ng/µl with **1x PP-02 Assay Buffer** (25 µl/well).
9. Add 25 µl of diluted **IgE-Biotin** to all wells.
10. Incubate at RT for 1 hour with gentle agitation.

	Blank	**Positive Control**	**Test Inhibitor**
1x Assay Buffer	20 µl	20 µl	20 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted IgE (0.2 ng/µl)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

11. Wash the plate three times with 200 µl of **PBST Buffer** per well and tap the plate onto clean paper towels.

Step 3: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with Blocking Buffer 3 (50 µl/well).
2. Add 50 µl of **diluted Streptavidin-HRP** to every well.
3. Incubate for 1 hour at RT.

4. Wash the plate three times with 200 µl of **PBST Buffer** per well and tap the plate onto clean paper towels.

5. Just before use, mix 1 volume of **ELISA ECL Substrate A** and 1 volume of **ELISA ECL Substrate B** (100 µl of mix/ well).

6. Add 100 µl of mix to every well.

7. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

8. The "Blank" value should be subtracted from all other values.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of controls.

Example Results

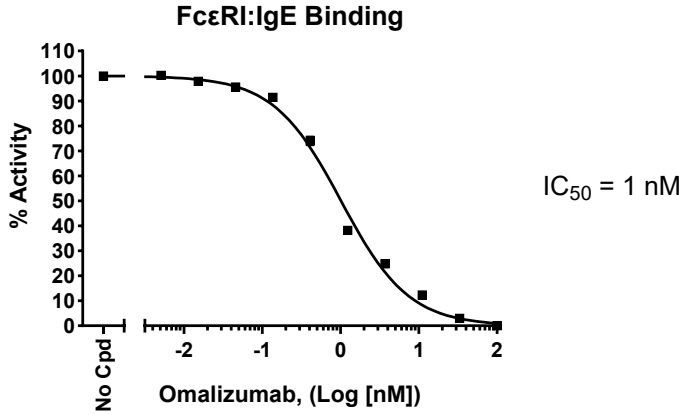

Figure 2: Inhibition of IgE binding to FcεRI by Omalizumab (Anti-IgE Antibody).
IgE was preincubated with increasing concentrations of Omalizumab. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as a percentage of binding activity in which the condition without antibody is set to 100%.

Data shown is representative.

References

Vogel M. and Engeroff P., 2024 *Antibodies (Basel)* 13(3):58.
Sutton B., *et al.*, 2019 *Antibodies (Basel)* 8(1):19.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

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