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Diagnostik & molekulare Diagnostik



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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Human IgE Chemiluminescent ELISA Kit is designed to detect and quantify protein levels of human IgE (immunoglobulin E) captured by pre-matched antibody pairs. This assay kit comes in a convenient 96-well format, with a pre-coated anti-IgE-specific antibody plate, enough recombinant purified IgE standard, and detection reagents for 100 wells.

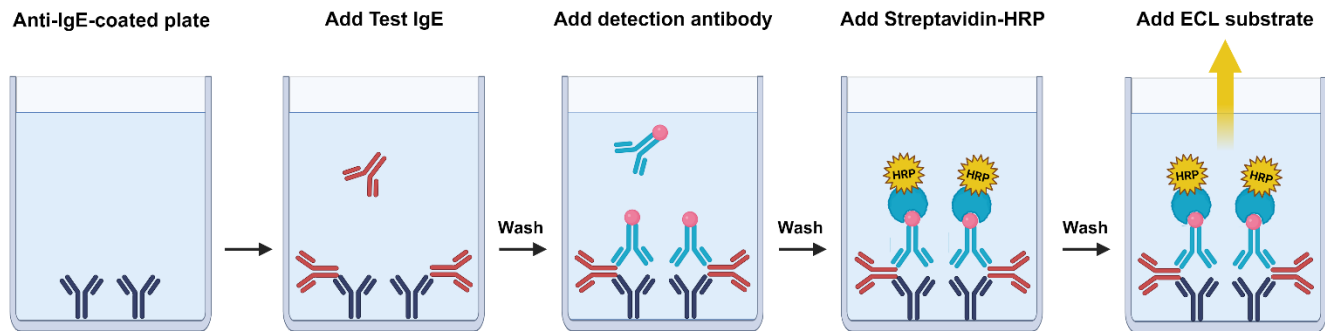


Figure 1: Human IgE Chemiluminescent ELISA Kit assay principle.

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 (rheumatoid 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. In addition, the properties of IgE can be used to increase ADCC (antibody-dependent cell mediated cytotoxicity) by monocytes and other immune cells, ADCP (antibody dependent cell-mediated phagocytosis) and other mechanisms that target cancer cells.

Applications

Quantify human IgE in cell culture supernatants.

Supplied Materials

Catalog #	Name	Amount	Storage
	96-well Anti-IgE Pre-Coated Plate	1	+4°C
82723	Anti-IgE Detection Antibody	6 µl	-80°C
82724	Streptavidin HRP	6 µl	-80°C
79743	Blocking Buffer 3	25 ml	+4°C
82722	IgE Standard*	3 µg	-80°C
79670	ELISA ECL Substrate A	6 ml	Room Temp
	ELISA ECL Substrate B	6 ml	Room Temp
	Adhesive plate seal	1	Room Temp

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

Materials Required but Not Supplied

- Test Samples
- PBST Buffer (1x PBS with 0.05% Tween-20)
- Diluent Solution (e.g. cell culture medium like DMEM (Dulbecco's Modified Eagle Medium))
- 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

The Human IgE Chemiluminescent ELISA 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 “Negative Control”, “IgE Standard”, and “Test Sample” conditions.
- We recommend maintaining the diluted antibody on ice during use.
- Variation in sample collection, processing and storage may cause differences in sample assay results.

- We recommend using IgE Standard (#82722) as internal control. If not running a full standard curve, we recommend running the IgE standard at 0.1X, 1X and 10X the EC₅₀ value shown in the validation data below.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend adding protease inhibitors (#82199) to samples and store samples at -80°C to avoid loss of bioactive human IgE.
- Samples containing a visible precipitate must be clarified prior to use in the assay.
- Avoid repeated freeze-thaw cycles. The frozen sample should be thawed on ice and mixed gently.
- The linear range of the assay is: 0-200 ng/ml.

Step 1: IgE Binding

1. Rehydrate the plate by adding 200 µl of PBST to every well.
2. Incubate 15 minutes at Room Temperature (RT).
3. Remove the PBST and tap the plate onto clean paper towels to remove all liquid.
4. Thaw the IgE Standard on ice. Briefly spin the tube to recover the full content of the tube.
5. Dilute the IgE Standard to 4 ng/µl (50 µl/well) in the same Diluent Solution that was used in sample preparation. This will correspond to the highest value on the standard curve.

Note: It is recommended to use the same buffer or medium as the Diluent Solution as the one used in the preparation of the "Test Sample". For example, if the uptake and recycling of human IgE antibodies by a pool of cells is analyzed, the same cell medium should be used as Diluent Solution to prepare an IgE standard. Alternatively, PBS or PP-02 buffer (BPS Bioscience, sold separately) can be used.

6. Prepare a serial dilution (1:3 recommended) of the diluted IgE Standard using the preferred diluent solution (50 µl/well).
7. Add 50 µl of Diluent Solution to "Negative Control" wells.
8. Add 50 µl of IgE Standard dilutions to wells labeled "IgE Standard".
9. Add 50 µl of each test sample to the wells labeled "Test Sample".
10. Incubate the plate at RT with slow agitation for 1 hour.
11. Wash the plate three times with 200 µl of PBST Buffer per well.

Step 2: Detection

1. Dilute Anti-IgE Detection Antibody 1000-fold in Blocking Buffer 3 (50 µl/ well).
2. Add 50 µl to each well.

3. Incubate at RT with shaking for 45 minutes to 1 hour.
4. Wash plate three times with 200 μ l of PBST Buffer per well.
5. Dilute Streptavidin HRP 1000-fold in Blocking Buffer 3 (50 μ l/ well).
6. Add 50 μ l to each well.
7. Incubate at RT with shaking for 30 minutes.
8. Wash plate three times with 200 μ l of PBST Buffer per well.
9. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 μ l of mix/ well).
10. Add 100 μ l of mix to every well.
11. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.
12. The “Negative Control” value should be subtracted from all other values.
13. If applicable generate a standard curve of luminescence versus IgE standard concentrations and determine concentration of the “Test sample”. For detailed information regarding standard curve and determination of the “Test sample” concentration refer to <https://bpsbioscience.com/assay-kits-faq>.

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 a control assay without enzyme (typically we set this value as 100).

Example Results

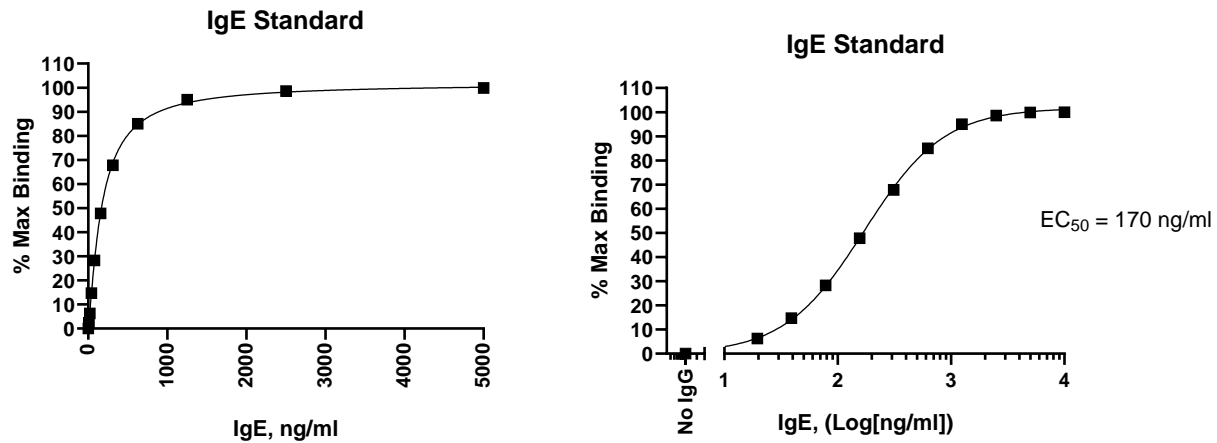


Figure 2. Example of IgE standard curves.
Various amounts of the IgE standard were run in duplicate.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

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 all further questions, please email support@bpsbioscience.com

Related Products

Products	Catalog #	Size
Human IgE Colorimetric ELISA Kit	82721	96 reactions
Human IgG Chemiluminescence ELISA Kit	82611	96 reactions
Human IgG Colorimetric ELISA Kit	82612	96 reactions
Anti-Human IgG, Unconjugated Antibody	100736	100 µg/ 500 µg
Anti-Human IgG, Unconjugated Antibody	100737	100 µg/ 500 µg

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