Human Immunglobulin E [IgE] ELISA Kit

PromoKine

Instruction Manual

Cat. No. PK-EL-K6511

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Intended Use

This PromoCell ELISA is intended for the quantitative determination of **IgE** in serum and stool. For *in vitro* research use only.

Summary and Explanation of the Test

Immunoglobulin E (**IgE**) has been demonstrated in several human secretions and fluids, such as respiratory tract mucus and saliva, urine, tears, human milk as well as in intestinal juices.

The **IgE** is responsible for various hypersensitivity reactions of the immediate type (Type-I-hypersensitivity). Polyvalent antigens trigger B-cells to produce specific **IgE** in large amounts. The released **IgE** antibodies bind to the Fc receptor on the surface of mast cells. The next time the sensitized person has contact to the allergen, it cross links the **IgE** on the surface of the mast cells and thereby triggers the mast cells to release histamine, which cause the various symptoms.

IgE is involved in a number of allergic phenomena such as erythema, anaphylaxis, homocytotropic histamine release, skin-sensitization and atopic reaction. In patients suffering from allergy, the **IgE** levels increase as much as three to four times compared to the healthy.

Indication

- Diagnosis of allergy and atopic disease in infants with relapsing, spastic bronchitis, pseudocroup, etc.
- Food allergy and intolerances
- Immunodeficiency syndromes
- Differential diagnosis of eczema, respiratory tract disease, rhinopathy, urticaria and other allergy related reactions

Material Supplied

Content	Kit Components	Quantity
PLATE	One holder with precoated strips	12 x 8
WASHBUF	ELISA wash concentrate 10x	2 x 100 ml
CONJ	Conjugate, (anti-IgE, Peroxidase-labeled)	1 x 150 µl
STD	Standards, ready to use (0; 0.62; 2.5; 10; 40 kU/l)	5 x 1 ml
CTRL 1	Control, ready-to-use	1 ml
CTRL 2	Control, ready-to-use	1 ml
SAMPLEBUF	Sample dilution buffer, ready-to-use	50 ml
SUB	Substrate (Tertramethylbenzidine), ready-to-use	1 x 15 ml
STOP	Stop solution, ready to use	1 x 15 ml

Material Required but not Supplied

- Ultrapure water*
- Stool sample application system
- Calibrated precision pipettors and 10-1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader

Preparation and Storage of Reagents

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. The kit can be used up to 4 times within the expiry date stated on the label.

- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.

- **Preparation of the wash buffer**: The **wash buffer concentrate (WASHBUF)** has to be diluted with ultra pure water **1:10** before use (100ml WASHBUF + 900ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37°C. The **WASHBUF** is stable at **2–8°C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8°C for 1 month.**

- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in wash buffer (100µl CONJ + 10ml wash buffer). The CONJ is stable at **2–8°C** until the expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **is not stable and cannot be stored.**

- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8°C.**

Storage and Preparation of Samples

Serum

Serum samples must be diluted **1:50** before performing the assay,
e.g. **20µl** sample + **980µl** sample dilution buffer (SAMPLEBUF), mix well.

100µl of the dilution are used in the test.

Precautions

- All reagents in the kit package are for *in vitro* research use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

Extraction of the stool samples

Wash buffer (1:10 diluted WASHBUF) is used as a **sample extraction buffer**. We recommend the following sample preparation:

Stool Sample Application System (SAS)

Stool sample tube – Instructions for use

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

SAS with 0.75 ml sample extraction buffer:

Applied amount of stool: 15mg

Buffer Volume: 0.75ml

Dilution Factor: 1:50

Please follow the instructions for the preparation of stool samples using the SAS as follows:

a) The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.

b) Fill the **empty stool sample tube** with **0.75 ml sample extraction buffer** (1:10 diluted WASHBUF) before using it with the sample. **Important:** Allow the sample extraction buffer to reach room temperature.

c) Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15mg of sample to be diluted. Screw tightly to close the tube.

d) Shake the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with sample extraction buffer for ~ 10 minutes improves the result.

e) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.

f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution I: 1:50
For analysis, pipet **100µl** of **dilution I** per well.

Sample storage
Dilution I is stable for approximately 1 month at -20°C

Assay Procedure

Principle of the test

This ELISA is designed for the quantitative determination of IgE.

In a first incubation step, the IgE in the samples is bound to polyclonal antibodies (in excess), which are immobilised to the surface of the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, a peroxidase-labelled anti-IgE (POD-Antibody) antibody (polyclonal) is added. After another washing step, to remove all unbound substances, the solid phase is incubated with the substrate, Tetramethylbenzidine (TMB). An acidic stop solution is then added to stop the reaction. The colour converts from blue to yellow. The intensity of the yellow colour is directly proportional to the concentration of IgE. A dose response curve of the absorbance unit (optical density, OD at 450nm) vs. concentration is generated, using the values obtained from the standard. IgE, present in the patient samples, is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30°C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips covered at 2–8° C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact us via info@promokine.info.

We recommend carrying out the tests in duplicate.

Before use, wash the wells **5 times** with **250µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.

1. Add each **100µl standards/controls/diluted samples** into the respective wells.
2. Cover the strips and incubate for **1 hour** at room temperature (15-30°C) on a **horizontal shaker***.
3. Discard the content of aech well and wash **5 times** with **250µl wash buffer**. After the final washing step, remove resicual wash buffer by firmly tapping the plate on absorbent paper.
4. Add **100µl conjugate** (diluted CONJ) into each well.
5. Cover the strips and incubate for **1 hour** at room temperature (15-30°C) on a **horizontal shaker***.
6. Discard the content of aech well and wash **5 times** with **250µl wash buffer**. After the final washing step, remove resicual wash buffer by firmly tapping the plate on absorbent paper.
7. Add **100µl substrate** (SUB) into each well.
8. Incubate for **5-10min**** at room temperature (15-30°C) in the **dark**.
9. Add **100µl stop solution** (STOP) into each well and mix well.
10. Determine **adsorption immediately** with an ELISA reader at **450nm** against 620nm (or 690nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, adsorption must be measured immediately at **405nm** against 620nm as a reference.

* We recommend shaking the strips at 550rpm with an orbit of 2mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

Results

The following algorithms can be used alternatively to calculate the results. We recommend using the 4-parameter algorithm.

1. 4 parameter-algorithm

   It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. Point-to-point calculation

   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline algorithm

   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Serum and stool sample

The obtained results have to be multiplied with the **dilution factor of 50** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

Limitations

Samples with an OD higher than the OD of the highest standard should be further diluted and re-assayed. For the following analysis, the changed dilution factor has to be taken into consideration.

Quality Control

PromoCell recommends the use of external controls for internal quality control, if possible.
Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

IgE (serum): < 100 kU/l

We recommend each laboratory to establish its own reference range.

General Notes on the Test and Test Procedure

- The quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. PromoCell GmbH can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be sent to PromoCell GmbH along with a written complaint.

Technical Hints

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

Ordering Information

Product Name	Product Description	Size	Catalog Number
Immunglobulin E [IgE] ELISA Kit, human	Human Immunglobulin E [IgE] ELISA Kit	96 Tests	PK-EL-K6511

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

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