SPECTRUM

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AllergenP Total IgE ELISA

REF:1318 001 96 test

Intended Use

Microplate based ELISA (enzyme linked immunosorbent assay) for the quantitative determination of Total immunoglobulin E in non hemolyzed human serum.

Background

Normally IgE concentration in serum is very low. It increases gradually from birth to teen-age. In adults normal concentration of IgE may reach 100 IU/mL. In elderly people IgE level sometimes decreases.IgE production is essential in anti-helminthic immunity.15-20-fold increase in IgE concentration is observed in the case of ascariasis. But in industrialized countries detection of high IgE concentrations is mainly connected with allergic diseases.Quantitative determination of total IgE has a great prognostic value.Detection of high IgE concentrations in serum by enzyme immunoassay is an important tool for differentiation between allergic diseases and other pathologies with similar clinical manifestations (such as asthma, frequent respiratory diseases, chronic rhinitis and dermatitis).Increased concentration of total IgE in serum was also reported in patients with lymphosarcoma and Hyper-IgE syndrome.

Assay Principle

This assay is based on the immunoenzymatic capture system,using the solid phase - wells of microstrips sensitized with human anti-IgE antibodies .During the first incubation the anti-IgEs in solid phase capture the IgEs of the sample.After an initial wash to eliminate any possible interference from other immunoglobulins, anti-IgE-Biotin conjugate is added to form solid phase|| Anti IgE: IgE: Anti IgE-biotin sandwich. After the wash the streptavidin-peroxidase conjugate is added and reacts with anti IgE-biotin conjugate.The last wash eliminates the unreacted species. A substrate (TMB solution) is added resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance. The color intensity is proportional to the amount of IgE in the sample. IgE concentration in the patient sample is read from a standard curve that is processed in each assay.

Reagents

Microplate (MP)
12 breakable 8 wells plate (Total 96 wells) coated with anti-IgE monoclonal antibodies

Sample diluent

Assay buffer
Saline solution

Enzyme Conjugate 1
Solution containing anti-IgE monoclonal antibodies conjugated with biotin

Enzyme Conjugate 2
Solution containing streptavidin conjugated with HRP

IgE Calibrators
Protein-based solutions containing known IgE concentrations. Exact concentrations are printed on the vial label.

Substrate (TMB solution): 3,3',5,5'-tetramethyl benzidine solution in citrate buffer containing hydrogen peroxide

Wash Solution Concentrate
Wash solution P, 20X concentrated:surfactant in buffered saline

Stop Solution
1 N HCl solution

Materials Required but not Provided

1- Automatic pipettes 2- Incubator
3- Equipment for rinsing wells 4- Elisa reader 5-Vortex tube mixer.
6- Disinfectant 7- Adsorbent material

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in-vitro diagnostic use		Use by/Expiration Date
LOT	Batch Code/Lot number		CAUTION. Consult instructions for use
REF	Catalogue Number		
	Consult instructions for use		Manufactured by

Reagents Storage and Stability

1- Avoid exposure to direct sunlight during incubations;
2- Kit is stored at 2-8°C and stable till the expirtation date.
After initial opening the kit is stable till expiration date if stored at 2-8 °C.
3- Wash solution prepared for use: at room temperature for 5 days

Reagent Preparation

1. Bring all reagents to room temperature (18-25 ^{O}C) prior to use for at least 30 minutes.
2. Liquid calibrators are ready to use.
3. Add 1 volume of Wash Solution Concentrate to 19 volumes of distilled water and mix well. Prepare the required volume according to the number of assayed samples.
For example: Add 5ml of wash solution to 95ml of distilled water.
4- Protect substrate from direct light.

Specimen Collection and Preservation

1- Serum only is suitable for analysis. The only acceptable anticoagulants are heparin, EDTA and sodium citrate. Specimen should be promptly separated from cells after blood collection.

2- Cap and store the samples at 18-25 ^{O}C for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ^{O}C up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ^{O}C. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortexing or by inverting 10 times. After thawing, bring to room temperature and mix well by gently shaking.

Precautions and warnings

1. Despite being confirmed as negative to all viruses, It is recommended that all human sourced controls be considered potentially infectious.

2. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.

3. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.

4. Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.

5. Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.

6. Do not touch or splash the rim of the well with conjugate.

7. Do not mix or use components from kits with different batch codes.

8. When manual pipette is used, complete pipetting of all controls, samples within 10 minutes.

9. It is important that the time of reaction in each well is held constant to achieve reproducible results.

10. The addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and Stop Solution should be added in the same sequence to eliminate any time deviation during reaction.

11. When handling conjugate vials, change gloves that have contacted human plasma/sera since introduction of human IgG/IgM will result in a neutralized conjugate.

Assay Procedure

1. Pipette 150 µL of Assay buffer into each well, except well A1(blank).
2. Pipette 25 µL of calibrators or samples .
3. Incubate for 60 minutes at +37 °C
4. Wash wells 3 times
5. Pipette 100 µL of Enzyme conjugate 1 into each well (except blank well)
6. Incubate for 30 minutes at 37°C
7. Wash wells 3 times
8. Pipette 100 µL of Enzyme conjugate 2 into each well (except blank well)
9. Incubate for 30 minutes at 37°C
10. Wash 3 times
11. Pipette 100 µL of substrate into each well
12. Incubate for 15 minutes at room temperature in the dark
13. Pipette 100 µL of stop solution to each well and shake for 1-2 minutes at room temperature
14. Read absorbance at 450 nm.

Assay Scheme	
Assay buffer	150 µl
Calibrators,or sample	25 µl
Incubation time	60 minutes
Washing step	3 times
Enzyme conjugate 1	100 µl
Incubation time	30 minutes
Washing step	3 times
Enzyme conjugate 2	100 µl
Incubation time	30 minutes
Washing step	3 times
Substrate	100 µl
Incubation time in dark	15 minutes
Stop solution	100 µl
Read absorbnace	450 nm

Wash procedure

1-Remove the contents of the wells into container with disinfectant;
2-Dispense 350 µL of wash solution, shake the plate carefully for 5–10 sec and remove the contents of the wells; repeat 3 times.
3-Strike the wells sharply on absorbent material to remove any liquid residue.

Expected Values:

It is recommended that each laboratory establishes its own reference range for the population of interest.The available literature provides the following informations relative to total IgE concentrations in human serum.

Age (years)		IU/ml
newborns - 1	less than	15
1- 7	less than	50
7-14	less than	100
Adults	less than	150

Quantitative determination

Specialized software for quantitative determination is recommended. Mean Absorbance of calibrators are plotted versus their respective IgE concentrations using 4PL or 5PL fit (see typical standard curve, fig.1).Calculate concentration of IgE in samples using standard curve. Any extrapolation of the standard curve to IgE above the nominal value of the calibrator 5 is forbidden. In this case the sample should be diluted 5- or 10 fold with sample diluent and re-tested. Multiply the measured concentration of pre-diluted samples by corresponding dilution factor.

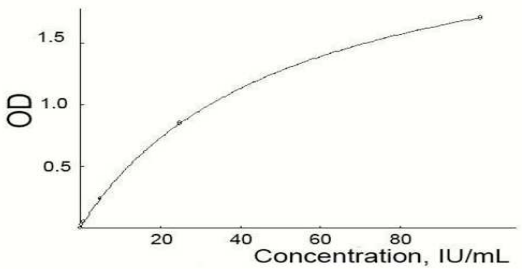

Figure 1: Typical example of standard curve at 450 nm

Data Reliability

The data should meet the following criteria:
- average blank OD (in well A1) < 0.09; If the data obtained do not meet the criteria, the results are considered unreliable and the test should be repeated.

Limitations

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. For diagnosis establishment, a physician is supposed to consider all available clinical and laboratory findings.

Analytcal Sensitivity

Analytical sensitivity of **AllergenP Total IgE,** i.e. the lowest detectable concentration that can be distinguished from the patients' sample, is 3.9 IU/mL. It was defined as mean of 20 replicates of Calibrator 0 plus 2 SD.

Specificity

No cross-reaction of monoclonal antibodies to IgE was detected with IgA, IgG, IgM and IgD.

Performance Charateristics

Measurement pricision

Within run (Repeatability)

With one human serum based panel, using one batch of reagent in replicates of 100 samples data are as following:

Mean = 17.9 **SD =** 0.065 **CV =** 4.2 %

Run to run (Reproducibility)

With one human serum based panel, using one batch of reagent in replicates of 100 samples across 3 separate runs data are as following:

Mean =20.7 **SD =** 0.021 **CV =** 7.56 %

References

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