RESEARCH AND PRODUCTION COMPANY
DIAGNOSTIC SYSTEMS Ltd.
RPC « Diagnostic Systems» Ltd**,** 22, Yablonevaia Street, 603093 Nizhni Novgorod, Russian Federation
Tel/fax: (831) 434 97 70, E-mail: info@npods.ru

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INSTRUCTIONS FOR USE
"DS-EIA-IgE TOTAL"
ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF TOTAL IMMUNOGLOBULIN E CONCENTRATION

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This Package Insert provides information for Professional Use of the kit.
Format 1 contains sufficient reagents for 96 (break apart plate) assays including controls; partial use of the kit is possible; (can be used for manual protocol).

I. INTENDED USE

The "DS-EIA-IgE TOTAL" kit is intended for the quantitative determination of total immunoglobulin E (IgE) concentration in human serum (plasma) by a microplate immunoenzymometric assay.

For ***In Vitro*** Diagnostic Use.

II. INTRODUCTION

IgE is the class of immunoglobulins, which are found out in norm in insignificant quantities in whey of serum and secrets (less than 0.001 % from all immunoglobulins of serum). According to WHO 1 IU corresponds to 2.4 ng. The newborn level of the total IgE is less than 1 IU/ml. IgE levels show a slow increase during childhood, reaching adult levels in the second decade of life. In general, elevated levels of IgE indicate an increased probability of an IgE-mediated hypersensitivity, being responsible for allergic reactions. However it is necessary to mean that approximately at 30 % of patients with atopic manifestations can have normal level of the total IgE; on the contrary, raised IgE levels can be revealed at a person without an allergy.

These substances cause smooth muscle constriction and lead ultimately to allergic conditions such as wheal and flare reactions, hives, dermatitis, rhinitis, hay fever, asthma and anaphylactic shock. Infants and children with family history of atopic allergy are at increased risk of developing disease and constitute a prime population for screening.

Significant elevations may be seen in the sensitized individuals, but also in cases of myeloma, pulmonary aspergillosis, and during the active stages parasitic infections.

III. PRINCIPLE OF THE TEST

The "DS-EIA-IgE TOTAL" is a one-step immunoassay, based on principle of "sandwich" method. The assay utilizes two high affinity and specificity monoclonal antibodies (enzyme conjugated and immobilized), that can bind to two different epitopes on the intact IgE molecule. The sample is allowed to react simultaneously with these two antibodies, resulting in the IgE molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed with washing solution to remove unbound labeled antibodies. A solution of TMB-Substrate is added and incubated, resulting in the development of a blue color. The color development is stopped with the addition of Stopping Reagent, changing the color to yellow. The

concentration of IgE is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

IV. CONTENT OF THE KIT "DS-EIA-IgE TOTAL"

Table 1

LABEL	NATURE OF THE REAGENTS	PRESENTATION
Anti-IgE-coated microtiter wells	One 96-well (break apart) plate coated with monoclonal anti-IgE antibodies. Once opened, the microtiter wells should be used until expiration date. Store at 2-8 ºC.	1 plate
Calibrators	Six vials of human serum based reference calibrators for IgE total. **Approximate*** concentration: 0; 62.5; 125; 250; 500; 1000 IU/ml. The calibrators were calibrated using a WHO 2st International Standard 75/502 Transparent or slightly opalescent yellow color liquids. Contain 0.05 % Proclin 300, 0.004 % gentamycin sulfate, 0.1% phenol as a preservative. Once opened, the calibrators should be used within one month. Store at 2-8 ºC in a tightly sealed vials. * Exact levels are given on the labels.	6 vials, 0.3 ml (Calibrator 0 IU/ml – 2.0 ml)
Control serum	Control, human serum with a defined quantity of total IgE based. Refer to vial label for acceptable range. Transparent or slightly opalescent yellow color liquid. Contain 0.05 % Proclin 300, 0.004 % gentamycin sulfate, 0.1% phenol as a preservative. Once opened, the control should be used within one month. Store at 2-8 ºC in a tightly sealed vial.	1 vial 0.5 ml
Conjugate	Monoclonal anti-IgE antibodies conjugated to horseradish peroxidase. Transparent or opalescent pink color liquid. Contain 0.05 % Proclin 300, 0.004 % gentamycin sulfate, 0.1% phenol as a preservative. Once opened, the conjugate should be used within one month. Store at 2-8 ºC in a tightly sealed vial.	1 vial 15.0 ml
Washing Solution (concentrated 25-fold)	Transparent or slightly opalescent liquid, colorless, or pale yellow. Store at 2-8 ºC until expiration date in a tightly sealed vial.	1 vial 50.0 ml
TMB-Substrate	Tetramethylbenzidine in Citric acid buffer, containing H_2O_2. Transparent colorless liquid. Once opened, the TMB should be used within one month. Store at 2-8 ºC in a tightly sealed vial.	1 vial 14.0 ml
Stopping Reagent	0.2 M sulphuric acid solution. Transparent colorless liquid. Store at 2-8 ºC until expiration date in a tightly sealed vial.	1 vial 25.0 ml

Additionally the following may be included in the delivery set:
- a lid for polystyrene 96-well plates or a protective film for EIA plates;
- disposable tips;
- a plastic dish for liquid reagents;
- a self-sealing plastic bag.

V. PRECAUTIONS

The reliability of the results depends on correct implementation of the following Good Laboratory Practices:

- The temperature in the lab should be 18-24 ºC.
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.
- Before use, it is necessary to wait 30 minutes for the reagents to stabilize to room temperature (18-24 ºC).
- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugates.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- Use a new distribution tip for each sample.
- Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute conjugate and color development solution.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay's procedure.
- Use high quality water.
- Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

VI. HEALTH AND SAFETY INSTRUCTIONS

- All reagents included in the kit are intended for "in vitro diagnostic use".
- Human origin material used in the preparation of Control serum has been tested and found negative for HBsAg, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2), antigen p24 HIV-1.
- Because no known test method can offer complete assurance that infections agents are absent, handle reagents and patients samples as if capable of transmitting infections disease.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Do not pipette by mouth.
- Any equipment directly in contact with specimens and reagents as well as washing solutions should be considered as contaminated products and treated as such.
- Wear lab coats and disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- Avoid spilling samples or solutions containing samples.

- Avoid any contact of the TMB-Substrate and the Stopping Reagent with the skin and mucosa.
- Provide adequate ventilation.
- Do not forget to neutralize and/or autoclave the washing wastes or any fluids containing biological samples before discarding them into the container. Samples and reagent of human origin, as well as, contaminated material and products must be discarded after decontamination: either by immersion in bleach at a final concentration of 5% of sodium hypochlorite (1 volume of bleach for 10 volumes of contaminated fluid or water) for 30 minutes. Also solid wastes should be disinfected by autoclaving for 1 hour at temperature 124-128 ºC and pressure 1.5 kHz/sm^2 (0.15 MPa). Also liquid wastes can be disinfected by boiling treatment for 30 min or by autoclaving for 1 hour at temperature 124-128 ºC and pressure 1.5 kHz/sm^2 (0.15 MPa). Tools and equipment should be wiped 2 times by 70 % ethanol before and after work.
- Some reagents contain ProClin 300 (0.05 %).

Irritant. May cause sensitization by skin contact. After contact with skin, wash immediately with plenty of soap and water.

VII. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED WITH THE KIT:

- Purified water.
- Pipettes, single-channel and multi-channel, adjustable or preset, to measure and dispense 20 µl, 100 µl, 150 µl.
- Disposable pipette tips.
- Thermoshaker at (37.0 ± 0.5) ºC.
- Automatic microplate washer.
- Microplate reader equipped with 450 nm filters.
- Disposable gloves.

VIII. COLLECTION AND HANDLING OF SPECIMENS

Collection of blood samples should be implemented according to the current practices. Serum or plasma (EDTA-, heparin- or citrate plasma) can be used in this assay. Separate serum as soon as possible to avoid any hemolysis. Extensive hemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior to testing. Suspended fibrin particles or aggregates may yield falsely positive results. Do not heat the samples. For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

Samples can be stored at 2-8 ºC not more than for 72 hours; they may be deep-frozen at -20 ºC. Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrosted more than 1 time cannot be used. Samples with expressed hemolysis, hyperlipidemia and which were preserved by sodium azide must not be analyzed.

IX. PREPARATION OF THE REAGENTS

1. **Ready to use reagents:**
   - Anti-IgE-coated microtiter wells
   - Calibrators
   - Control serum
   - Conjugate
   - TMB-Substrate
   - Stopping Reagent

2. **Reagents to prepare:**
   Working Washing Solution. Thoroughly shake washing solution concentrate. To make Working Washing Solution take required amount of concentrate and mix with purified water (1:25 ratio) in a separate vial. Thoroughly mix the solution. The prepared Working Washing Solution is stable at least for 14 days at 18-24 ºC or for 28 days at 2-8 ºC when used in GLP condition.

X. TEST PROCEDURE

Note: Before use, allow reagents to reach room temperature (18 – 24 ºC) for 30 min.

1. Bring all components and clinical specimens to be tested to room temperature.
2. Format the microplate wells for each Calibrators, Control serum and patient specimens to be assayed in duplicate, add one or two wells for TMB control (blank). Replace any unused microwell strips back into the aluminum bag with silica gel drier and then put into the self-sealing plastic bag, seal and store at 2 - 8 ºC until expiration date.
3. Pipette 20 µl of each Calibrators, Control serum and samples with new disposable tips into appropriate wells. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
4. Add 150 µl of Conjugate into each well, except blank.
5. Cover the strips and incubate on a thermoshaker (approximately 500-800 rpm) for 45 minutes at (37.0 ± 0.5) °C.
6. Wash the wells 5 times with 300 µl of Working Washing Solution per well and tap the plate firmly against absorbance paper to ensure that it is dry (The use of a washer is recommended).
7. Pipette 100 µl of TMB-Substrate into each well at timed intervals.
8. Incubate for 20 minutes at room temperature in a dark.
9. Add 150 µl of Stopping Reagent into each well at the same timed intervals as in step 7. Gently mix for 10 seconds.
10. Read the plate on microplate reader at 450 nm within 20 minutes after addition of the Stopping Reagent. In case of overflow absorbance values, read at 405 nm.
Scheme of the assay is represented in Annex.

XI. CALCULATION OF RESULTS

1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the “blank” from the mean absorbance values of the calibrators, control and serum (plasma) samples.

4. Draw a calibrator curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.
5. Read the values of the unknowns directly off the calibrator curve.
6. If a sample reads more than 1000 IU/ml, then dilute it with calibrator 0. The result obtained should be multiplied by the dilution factor.

Typical tabulated data

Calibrator	OD1	OD2	Mean OD-blank	Value (IU/ml)
0	0.054	0.054	0	0
1	0.351	0.348	0.296	62.5
2	0.612	0.628	0.566	125.0
3	1.074	1.104	1.035	250.0
4	1.796	1.865	1.777	500.0
5	2.606	2.541	2.520	1000.0
Unknown	0.865	0.862	0.810	183.5

This data is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1**. Blank OD:** The absorbance value should not be more than 0.1 at 450 (405) nm.
2. The absorbance (OD) of **Calibrator 5** should not be less than 1.3.
3. Calculated Value of **Control serum** should be within established range.

XII. PERFORMANCE CHARACTERISTICS OF "DS-EIA-IgE TOTAL"

1. Assay Dynamic Range

The range of the assay is between 0-1000 IU/ml.

2. Analytical sensitivity

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator 0 (based on 12 replicates) plus 2 SD.
Therefore, the sensitivity of the "DS-EIA-IgE TOTAL" kit is 99,95%**.**

3. Specificity (cross-reactivity)

No cross-reactivity to human IgA, IgG, IgM exists with this assay. The specificity of the "DS-EIA-IgE TOTAL" kit is 100%**.**

4. Precision

Intra-assay precision

The within assay variability is shown below:

Sample	n	Mean, IU/ml	SD	CV,%

1	9	183	7.45	4.1

Inter-Assay precision
The between assay variability is shown below:

Sample	n	Mean, IU/ml	SD	CV,%
1	27	178.2	12.18	6.8

5. Recovery
Spiked samples were prepared by adding defined amount of IgE to patient serum sample. The results (in IU/ml) are tabulated below:

Added Concentration, IU/ml	Measured Conc., IU/ml	Expected Conc. IU/ml	Recovery, %
-	183		
62.5	126	122.5	103

6. Linearity
Patient serum sample were diluted with calibrator 0. The results (in **IU/ml**) are tabulated below:

Undiluted	Dilution	Measured Conc., IU/ml	Expected Conc., IU/ml	Recovery, %
1000	1000:2	474.5	500	95
500	500:2	236.7	250	95
250	250:2	126.7	125	101

7. Expected normal Value
The total Immunoglobulin E level in normal, allergy-free adults is less than 190 IU/ml in the serum (plasma).

Each laboratory should establish its own normal ranges based on patient population in the geographical areas served.

8. High dose hook effect
The assay was tested for a high dose hook effect. Up to a t IgE concentration of 4000 IU/ml no hook effect was observed.

9. Accuracy
The “DS-EIA-IgE TOTAL” test was compared with an Enzyme immunoassay as a reference test. The total number of specimens was 266. The values ranged from 0 to 4669 IU/ml. The least square regression equation and the correlation coefficient were computed for “DS-EIA-IgE TOTAL” in comparison with the reference test. The least square regression analysis was y=1.015(x) +14.615 with correlation coefficient 0.97.

XIII. LIMITS OF THE TEST

1. All the reagents within the kit are calibrated for the direct determination of IgE in human serum (plasma). The kit is not calibrated for the determination of IgE in saliva or other specimens of human or animal origin.
2. Any improper handing of samples or modification of this test might influence the results.
3. Only calibrator 0 may be used to dilute any high serum (plasma) samples. The use of any other reagent may lead to false results.
4. The results obtained with this kit should never be used as the sole basis for clinical diagnosis. Any laboratory result is only a part of the total clinical picture of the patient.
5. Serum (plasma) samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
6. Some individuals may have heterophilic antibodies to mouse or other animal proteins that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

XIV. CONDITIONS OF STORAGE AND TRANSPORTATION

Expiry date is indicated on the packaging. Keep in dark dry place at 2-8 °C.
Transportation may be done by all kinds of covered transport at temperature 9-20 °C not more than during ten (10) days. Freezing is prohibited.

RPC Diagnostic Systems» Ltd
22, Yablonevaia Street
603093 Nizhni Novgorod
Russian Federation
Tel/fax: (831) 434 97 70
E-mail: info@npods.ru

XV. REFERENCES

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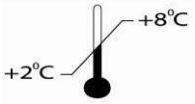
XVI. EXPLANATION OF SYMBOLS

CE	CE marking (European directive 98/79/CE on in vitro diagnostic medical devices)

	For in vitro diagnostic use
	Manufacturer
	Catalog number
	Sufficient for
	Lot code
	Storage temperature limitation
	Expiry date CCYY-MM-DD
	Consult Instruction for use
	Contains irritant agent

Annex

Scheme of the assay

1	**Add**	20 µl of Calibrators, Control serum
2	**Add**	20 µl of tested samples
3	**Add**	150 µl of Conjugate
4	**Incubate**	45 min, thermoshaker, 500-800 rpm, at (37.0 ± 0.5) °C
5	**Wash the plate**	Working Washing Solution, not less than 300 µl, 5 times
6	**Add**	100 µl of TMB-Substrate
7	**Incubate**	20 min, at 18-24 ºC in a dark place
8	**Add**	150 µl of Stopping Reagent
9	**Read the optical density**	450 (405) nm