

# PATHOZYME<sup>®</sup> IMMUNOGLOBULIN E <sup>Ref</sup> OD417

## Enzyme-Immunoassay (EIA) for the Quantitative Determination of IgE in human serum

Store at 2°C to 8°C. DO NOT FREEZE

For in-vitro use only.

### INTRODUCTION

Patients with atopic allergic diseases such as atopic asthma, atopic dermatitis and hay fever, have been shown to exhibit increased total Immunoglobulin E (IgE) levels in blood. IgE is also known as the reagenic antibody. In general, elevated levels of IgE indicate an increased probability of an IgE-mediated hypersensitivity, responsible for allergic reactions. Parasitic infestations such as hookworm and certain clinical disorders including aspergillosis, have also been demonstrated to cause high levels of IgE. Decreased levels of IgE are found in cases of hypogammaglobulinemia, autoimmune diseases, ulcerative colitis, hepatitis, cancer and malaria. Cord blood or serum IgE levels may have prognostic value in assessing the risk of future allergic conditions in children.

Certain groups of white blood cells, including basophils and tissue mast cells, have membrane receptors for the IgE molecule. These target cells, through a series of complex reactions, form a combination of a specific allergen with antibody-sensitised basophils such as histamine, into the blood stream. As a result of these biochemical mediators, there is a constriction of smooth muscles, dilation of small blood vessels, activation of blood platelets and irritation of skin nerve endings characteristic of allergic reactions.

Typical clinical symptoms of immediate hypersensitivity are inflammation and itching in a skin reaction or congestion in a bronchial reaction.

The IgE serum concentration in a patient is dependent on both the extent of the allergic reaction and the number of different allergens to which the individual is sensitised.

Non-allergic normal individuals have IgE concentrations that vary widely and increase steadily during childhood, reaching their highest levels at age 15 to 20 and thereafter remaining constant until about age 60, when they slowly decline.

### INTENDED USE

**PATHOZYME IgE** is an Enzyme Immunoassay (EIA) for the quantitative determination of Immunoglobulin E (IgE) in human serum.

For professional use only.

### PRINCIPLE OF THE TEST

Specific monoclonal anti-IgE antibodies are coated onto microtitre wells. Test sera are applied and incubated with the Zero Buffer. If human IgE is present in the sample, it will combine with the antibody on the well. The well is then washed to remove any residual test specimen and then IgE antibody, labelled with Horseradish Peroxidase enzyme (Conjugate), is added. This results in the IgE molecules being sandwiched between the solid phase and the enzyme linked antibodies. After incubation, the wells are washed with distilled water to remove unbound labelled antibodies. On addition of the Substrate (TMB), a colour will develop only in those wells in which enzyme Conjugate is present, indicating the presence of IgE. The enzyme reaction is stopped by the addition of dilute Hydrochloric Acid and the absorbance is then measured at 450nm. The concentration of IgE is directly proportional to the colour intensity of the test sample.

This test has been calibrated to WHO 2<sup>nd</sup> International Reference Preparation 75/502 (1981).

### CONTENTS

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Microtitre Plate		12 x 8 wells x 1
Breakable wells coated with specific antibody contained in a resealable foil bag with a desiccant.		
Cal A	0 IU / ml	0.5 ml
Reference Standard: BSA Buffer free of IgE Ready to use. (Colourless)		
Cal B	10 IU / ml	0.5ml
Reference Standard: IgE diluted in BSA Buffer. Ready to use. (Colourless)		
Cal C	50 IU / ml	0.5ml
Reference Standard: IgE diluted in BSA Buffer. Ready to use. (Colourless)		
Cal D	100 IU / ml	0.5ml
Reference Standard: IgE diluted in BSA Buffer. Ready to use. (Colourless)		
Cal E	400 IU / ml	0.5ml
Reference Standard: IgE diluted in BSA Buffer. Ready to use. (Colourless)		
Cal F	800 IU / ml	0.5ml
Reference Standard: IgE diluted in BSA Buffer. Ready to use. (Colourless)		
BUF	AS	11ml
Zero Buffer : Phosphate based buffer containing stabilising proteins. Ready to use. (Yellow)		
Conj		17 ml
Anti-IgE HRP Conjugate: Anti-IgE conjugated to HRP. Ready to use (Pink)		
Subs	TMB	11 ml
Substrate Solution: 3,3',5,5'-Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)		
Soln	Stop HCl	1M
Stop Solution: Hydrochloric Acid diluted in purified water. Ready to use. (Colourless)		

Instruction leaflet and EIA Data Recording Sheet 1 + 1

### MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes: 50µl, 100µl and 200µl  
Disposable pipette tips  
Absorbent paper  
Microplate reader fitted with a 450nm filter  
Graph paper  
Thoroughly clean laboratory glassware.

### PRECAUTIONS

**PATHOZYME IgE** contains materials of human origin which have been tested and confirmed negative for HCV, HIV 1 and II antibodies and HBsAg by FDA approved methods at single donor level. Because no test can offer complete assurance that products derived from human source will not transmit infectious agents it is recommended that the reagents within this kit be handled with due care and attention during use and disposal. All reagents should, however, be treated as potential Biohazards in use and for disposal. Do not ingest.

**PATHOZYME IgE** Reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation.

**PATHOZYME IgE** Stop Solution is dilute Hydrochloric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

**PATHOZYME IgE** contain 1% Proclin<sup>™</sup> 300\* as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with running water and seek medical advice.

\*Proclin<sup>™</sup> 300 is a trade mark of ROHM & HAAS Limited.

### STORAGE

Reagents must be stored at temperatures between 2°C to 8°C.

Expiry date is the last day of the month on the bottle and the kit label. The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

DO NOT FREEZE ANY OF THE REAGENTS as this will cause irreversible damage.

### SPECIMEN COLLECTION AND PREPARATION

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required.

Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversely affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at -20°C for up to 1 year. Thawed samples must be mixed prior to testing. Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.

Do not repeatedly freeze-thaw the specimens as this will cause false results.

### REAGENT PREPARATION

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

### LIMITATIONS OF USE

The use of samples other than serum has not been validated in this test. There is no reuse protocol for this product. When making an interpretation of the test it is strongly advised to take all clinical data into consideration. Diagnosis should not be made solely on the findings of one clinical assay.

## ASSAY PROCEDURE

1. Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assay.
2. One set of Standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA Data Recording Sheet provided.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.
4. Dispense 20µl of Standards and test serum into the assigned wells.
5. Dispense 100µl of Zero Buffer into each well.
6. Thoroughly mix for 30 seconds. It is very important to mix completely at this stage.
7. Incubate the plate for 30 minutes at room temperature (20°C to 25°C).
8. At the end of the incubation period, discard the contents of the wells by flicking plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the Biohazard container.
9. Hand Washing: Fill the wells with a minimum of 300µl of distilled water per well. Flick plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Wash the empty wells 5 times.
10. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
11. Machine Washing: Ensure that 300µl of distilled water is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty wells 5 times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
12. Dispense 150µl Anti-IgE HRP Conjugate into each well and mix gently for 10 seconds.
13. Incubate for 30 minutes at room temperature (20°C to 25°C).
14. Wash plate as above.
15. Dispense 100µl Substrate Solution into each well and mix gently for 5 seconds.
16. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
17. Stop the reaction by adding 100µl Stop Solution to each well.
18. Gently mix for 30 seconds to ensure that the blue colour changes completely to yellow colour.
19. Read the optical density immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.

## TROUBLESHOOTING

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

Use a separate disposable tip for each sample to prevent cross contamination.

Duplication of all standards and specimens, although not required, is recommended.

Specimens and standards should be run at the same time to keep testing conditions the same.

It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used, since pipetting of all Standards and specimens should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available. Replace caps on all reagents immediately after use.

Avoid repeated pipetting from stock reagents as this is likely to cause contamination.

Do not mix reagents or antibody coated strips from different kits. When dispensing, care should be taken not to touch the surface of the well.

Do not allow reagent to run down the sides of the well. Prior to the start of the assay bring all reagents to room temperature (20°C to 25°C). Gently mix all reagents by gentle inversion or swirling.

Once an assay has been initiated, the wells should not be allowed to become dry during the assay.

Do not contaminate the Substrate Solution as this will render the whole kit inoperative.

Check the precision and accuracy of the laboratory equipment used during the procedure to ensure reproducible results.

The unused strips should be resealed in the foil bag, containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.

## CALCULATION OF RESULTS

Calculate the mean absorbance value (A450) for each set of Standards and specimens. Construct a standard curve by plotting the mean absorbance from each Standard against its concentration in IU/ml on graph paper. Use the mean absorbance values for each specimen to determine the corresponding concentration of IgE in IU/ml from the standard curve.

If levels of controls or users known samples do not give expected results, test results must be considered invalid.

If using a software package choose a polygon with data extrapolation curve fit.

## EXPECTED VALUES AND SENSITIVITY

The graph produced by the Calibrators should be Hyperbolic in shape with the OD450 of the Calibrators proportional to their concentration. The OD of Calibrator A should be less than 0.75 and the OD of Calibrator F should be greater than 1.5 for the assay results to be valid.

Age	Normal ( allergy free IgE ) Levels IU/ml
0 . 4 days	< 1.5
5 days . 12 months	< 15
1 . 5 years	< 60
6 . 9 years	< 90
10 . 16 years	< 200
16 + years	< 100

The minimum detectable concentration of IgE by **PATHOZYME IgE** is estimated to be 5.0IU/ml.

## EVALUATION DATA

Calibrated to major competitors and in house standards.  
The co-efficient of variation of **PATHOZYME IgE** is less than or equal to 10%.

In an evaluation between the Omega Pathozyme IgE kit and the Hybridtech Tandem E IgE for samples with levels between 6.4 and 1039.2 IU/ml the following data was generated.

Number of Samples	98
Correlation Co-efficient	0.96
Slope	1.108
Intercept	- 23.8
Omega Mean	209.4 IU/ml
Hybridtech Tandem E IgE Mean	208.1 IU/ml

These kits were shown to give good correlation.

## REFERENCES

- (1) Zetterstrom and Johansson, S. G. O. *Allergy*. 1981;36:537.
- (2) Buckley, R. H. *Immunopharmacology of Allergic Disease*. 1979;1:17.
- (3) Michel, F. B., Bousquet, J. and Greillier, P. J. *Allergy Clin. Immunol.* 1980;64:422.
- (4) Ishizaka, T. *Ann. Allergy*. 1982;48:313.
- (5) Kulczyński, A. Jr. *J. Allergy Clin. Immunol.* 1981;68:5.

## QUICK REFERENCE TEST PROCEDURE

1. Dispense 20µl of Standards or test serum and 100µl of Zero Buffer into each well. Gently mix thoroughly for 30 seconds.
2. Incubate for 30 minutes at room temperature (20°C to 25°C).
3. Discard well contents and wash five times with distilled water.
4. Add 150µl Anti-IgE HRP Conjugate into each well and gently mix for 10 seconds.
5. Incubate for 30 minutes at room temperature (20°C to 25°C).
6. Discard well contents and wash five times with distilled water.
7. Add 100µl of Substrate Solution into each well and gently mix for 5 seconds.
8. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
9. Add 100µl of Stop Solution to each well and gently shake for 30 seconds.
10. Read the Optical Densities immediately (no later than 10 minutes) using microplate reader with a 450nm filter.

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