ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#**0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1800

Human Total IgE(IgE)

ELISA KIT Cat. No. 1800, 96 Tests

For Quantitative Determination of Total Human IgE In Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

Total Human IgE ELISA KIT Cat. No. 1800

For Quantitative Determination of Human IgE In Serum

Kit Contents: (reagents for 96 tests)

Components	
Streptavidin coated microwell strip Plate (96 wells), #1801	1 plate
IgE **Standard A** (0 IU/mL), 0.5 ml #1802A	1 vial
IgE **Standard B** (5 IU/mL), 0.5 ml #1802B	1 vial
IgE **Standard C** (25 IU/mL), 0.5 ml #1802C	1 vial
IgE **Standard D** (50 IU/mL), 0.5 ml#1802D	1 vial
IgE **Standard E** (150 IU/mL), 0.5 ml #1802E	1 vial
IgE **Standard F** (400 IU/mL), 0.5 ml # 802F	1 vial
Anti-h IgE HRP Conjugate, 12 ml #1804	1 bottle
IgE Biotin Conjugate, 12 ml # 1805	1 bottle
HRP substrate Solution, 12 ml # TMB1800	1 bottle
Wash buffer (20X), 25 ml, dilute 1:20 with distilled water # WB-1800	1 bottle
Stop solution, 12 ml #ST-1800	1 bottle
Complete Instruction Manual # M-1800	1

Intended use: ADI's Human IgE ELISA kit is intended for quantitative Determination of Total Human IgE In Serum. **For in vitro research use only (RUO).**

Introduction

IgE immunoglobulin is a four chain molecule with a mole. Wt. Of ~190 kDa. It is a unique class of immunoglobulin which is important in the mediation of the allergic response. The mechanism of action involves an initial antigenic stimulation of immunocompetent B lymphocytes by a specific antigen, a process which induces the lymphocytes to respond by producing specific antibodies of several classes. Once class of reaginic or IgE antibody, becomes partially bound via its Fc portion to receptors on the surface of mast cells and basophilic leukocytes. Upon further stimulation to specific allergens, these cell-bound IgE molecules bind via their Fab portion to the allergen. This combination triggers the mast cells and basophiles to release, various vasoactive amines into blood and immediately surround tissue. These agents include an increase in vascular permeability, an efflux of blood components and the consequent symptoms characteristics of an allergic reaction. Typical symptoms are inflammation and itching in the case of a bronchial reaction.

IgE determinations are valuable in the diagnostic assessment of patients with established or suspected allergic diseases. Studies have shown that conditions such as asthma, rhinitis, eczema, urticaria, dermatitis, and some parasitic infections lead to increased IgE levels.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on replicates determinations of the zero standard, the minimum concentration of human IgE detected using this assay is 5 IU/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Three serum samples were run in ten replicates in an assay. The samples showed good intra-assay precision (3.2-8 %CV). The actual values were: mean 16.5 IU/ml, %CV 3.2; mean 80 IU/ml, %CV 8; mean 168.5 IU/ml, %CV 5.8, respectively

Inter-assay precision:

Three serum samples were run in duplicate in eight independent assays. The samples showed good inter-assay precision (average 6 %CV). The actual values were: mean 19.3 IU/ml, %CV 6.5; mean 77.5 IU/ml, %CV 6; mean 154.5 IU/ml, %CV 6.5, respectively.

3. RECOVERY

A known amount of h IgE (50, 100, and 200 IU/ml) was added to two patient sera (with original IgE concentrations of 10 and 40 IU/ml) and the final total IgE concentrations measured. The assay showed excellent mean recoveries of about 102% (range 96-109%).

4. SPECIFICITY

The specificity of IgE kit was determined by measuring interference from high concentrations (up to 300 mg/ml) of human serum IgA, IgG, and IgM. No cross reactivity was observed (< 5 IU/ml).

5. HIGH DOSE HOOK EFFECT

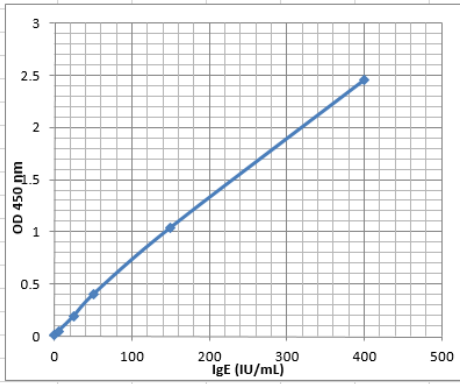
IgE concentrations of up to 5000 IU/ml did not cause any hook effect.

General References: Savanat T (1997) J. trop. med. Pub. Hlth. 8, 2, 149; Berg T (1978) Intl Arch. Allergy 36, 219, 1969; Bennich HH (1978) Immunol. Rev. 41, 261; Hamburger RN (1980) Immunol. Allergy Practive 2 6, 182; Johnsson SH (1972) Progress IN Clin. Immunol. 1972; Leonardy F (1972) Annals. Allergy 30, 378

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450nm}
A1, A2	**Std. A** (0 IU/mL)	0.009
B1, B2	**Std. B** (5 IU/mL)	0.046
C1, C2	**Std. C** (25 IU/mL)	0.192
D1, D2	**Std. D** (50 IU/mL)	0.398
E1, E2	**Std. E** (150 IU/mL)	1.041
F1, F2	**Std. F** (400 IU/mL)	2.458
G1, G2	**Sample 1**	

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values. Do not use this for calculating sample values)



*2015-kit-spec-XL

CALCULATION OF RESULTS

Calculate the mean Absorbance for each duplicate. Subtract the Absorbance of the zero standard from the mean Absorbance values of standards, control, and samples. Draw the standard curve on a graph paper by plotting net Absorbance values of standards against appropriate IgE concentrations. Read off the IgE concentrations of the control and patient samples. If ELISA reader software is being used, we recommend 4-paramter or 5-parameter curve.

PRINCIPLE OF THE TEST

IgE ELISA kit is based on simultaneous binding of human IgE (IgE) from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of IgE present in the sample. The reaction is terminated by adding stopping solution. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of IgE in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International IgE ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. IgE Standards have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20^{o}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

This kit has been optimized with serum samples. Plasma samples have been used by some researchers (see refs on page 4).

REAGENTS PPEPARATION

Dilute wash buffer (1:20) with distilled water (25 ml stock in 475 ml). Store at 4oC.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions.

HRP substrate buffer (solution A) and HRP substrate (solution B) should be colorless at the time of use. If solutions have turned light blue in color, these should be replaced. Do not expose these solutions to strong light during storage or use.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. **Dilute wash buffer (1:20) with distilled water (25 ml stock in 475 ml).**

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **25 ul** of standards, control, and serum samples into appropriate wells in *duplicate*. Immediately add **100 ul** of the **biotin conjugate** into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at 18-26 ^{o}C.
3. Aspirate and wash the wells **3 times** with 300 ul of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **100 ul** of Ab-enzyme conjugate into each well**.** Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at 18-26 ^{o}C.
5. Aspirate and wash the wells **3 times** with 300 ul of diluted wash buffer, as above.
6. Dispense **100 ul TMB substrate per well, m**ix gently for 5-10 seconds. Cover the plate and incubate for **10 minutes** at room temperature (blue color develops in positive wells)
7. Stop the reaction by adding **50 ul** of stopping soln the same timed intervals into each well. Mix gently for 5-10 seocnds. Blue color turn yellow.
8. Measure the Absorbance at **450 nm** using an ELISA reader within 15-30 minutes.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C.

Addition of the HRP substrate solution starts a kinetic reaction which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure Absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Serum samples containing more than 640 IU/ml IgE should be diluted with the zero standard (standard A), reassayed, and the results obtained should be multiplied by the appropriate dilution factor.

QUALTITY CONTROL

No contros are supplied with the kit. We typically use Lyphochekc Level 1-3 for quality control.
Level 1: 151.78 (range 119-237 IU/ml)
Level 2: 61.28.78 (range 41-81.4 IU/ml)
Level 3: 104.36 (range 72.1-143.0 IU/ml)

IgE may vary as a result of geographical distribution, diet and season of the year. It is recommended that each laboratory establish the expected values for its samples.

EXPECTED VALUES

1. Serum IgE may vary as a result of geographical distribution, diet and season of the year. It is recommended that each laboratory establish the expected values for its samples.

Species crossreactivity

The antibodies used in the kit are highly specific to human IgE. Some reactivity may exist with monkey IgE or other species. However, we have not rigorously established the species croosreactivity. IgE kits for mouse, rat and animals IgE are available from ADI.

(2) Citations of ADI's IgE ELISA kit #1800 (see web site for updated list)

Vallerskog T, 2006, Clinical Immunology, 122, 62-74, used human plasma samples for human IgE ELISA