Other ELISA kits available from ADI

Related Items

Catalog#	ProdDescription
6310	Mouse IgA ELISA Kit, 96 tests, Quantitative
6320	Mouse IgG ELISA Kit, 96 tests, Quantitative
6330	Mouse IgG1 ELISA Kit, 96 tests, Quantitative
6340	Mouse IgG2a ELISA Kit, 96 tests, Quantitative
6350	Mouse IgG2b ELISA Kit, 96 tests, Quantitative
6360	Mouse IgG3 ELISA Kit, 96 tests, Quantitative
6370	Mouse IgE ELISA Kit, 96 tests, Quantitative
6380	Mouse IgM ELISA Kit, 96 tests, Quantitative
6380-RS	Mouse IgM Reference Serum for ELISA (~500 ng/ml)
6390	Mouse Transferrin (Tf) ELISA Kit, 96 tests, Quantitative

6320-RDT-25 TruStrip RDT Mouse IgG Rapid Test cards, 10/pk

Human: BD-1, BD-2, BD-3 **and**: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgG1, IgG4, IgA, Insulin, NSE, CA125, CA199, CA242, PAP, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgM, Leptin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF

Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Monkey: IgM, IgG, IgA, CRP
Chicken: IgG, IgM, IgY, Ovalbumin
Rabbit: CRP, IgG
Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM
Cat: IgG, IgM
Goat: IgG

Instruction Manual No. M-6370

Mouse IgE ELISA Kit

Cat. No. 6370, 96 tests

For Quantitative Determination of Mouse Immunoglobulin E in Serum, plasma or other biological fluids

ALPHA DIAGNOSTIC INTERNATIONAL

India Contact:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@atzlabs.com
Web: www.atzlabs.com

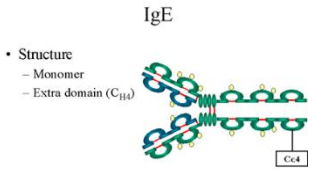
LIFE TECH Life Technologies™
Your Molecular & Cell Technology Partner

INTENDED USE

The Mouse IgE ELISA Kit is a sandwich ELISA for the quantification of IgE circulating in serum or in other appropriately qualified samples from tissue fluids (e.g., saliva, mucosa), or in cultures of mouse cells. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

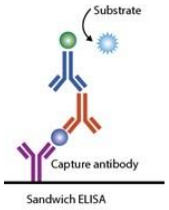
RESEARCH USE OF THE TEST

Immunoglobulin E (IgE) is a kind of antibody (or immunoglobulin (Ig) "isotype") that has only been found in mammals. Monomers of IgE consist of two heavy chains (ε chain) and two light chains, with the ε chain containing 4 Ig-like constant domains (Cε1-Cε4). IgE's main function is immunity to parasites such as helminths like Schistosoma mansoni, Trichinella spiralis, and Fasciola hepatica. IgE is utilized during immune defense against certain protozoan parasites such as Plasmodium falciparum. IgE also has an essential role in type I hypersensitivity, which manifests various allergic diseases, such as allergic asthma, most types of sinusitis, allergic rhinitis, food allergies, and specific types of chronic urticaria and atopic dermatitis. IgE also plays a pivotal role in responses to allergens, such as: anaphylactic drugs, bee stings, and antigen preparations used in desensitization immunotherapy. Although IgE is typically the least abundant isotype—blood serum IgE levels in a normal ("non-atopic") individual are only 0.05% of the Ig concentration,[8] compared to 75% for the IgGs at 10 mg/ml, which are the isotypes responsible for most of the classical adaptive immune response—it is capable of triggering the most powerful inflammatory reactions.



IgE is a unique class of immunoglobulin which is important in the mediation of allergic responses. The mechanism of action involves an initial antigenic stimulation of immunocompetent B lymphocytes by specific antigen, a process that induces the lymphocytes to respond by producing specific antibodies of several classes. The IgE class binds to receptors on the surface of mast cells. Upon further stimulation by specific allergens (antigens), the cell-bound IgE binds the allergen and, in combination with mast cells and basophiles, releases vasoactive amines into blood and surrounding tissue. These agents increase vascular permeability, an efflux of blood components and the consequent symptoms characteristic of allergic reaction. Studies have shown that conditions such as asthma, rhinitis, eczema, urticaria, dermatitis, and some parasitic infections lead to increased IgE levels. Mouse studies are an important mode of research regarding the mechanisms and treatment of allergic responses, and quantification of IgE levels is an important testing parameter.

PRINCIPLE OF THE TEST



The Mouse IgE ELISA kit is based on the binding of mouse IgE in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of IGE present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of IgE in samples and control is calculated from a curve of standards containing known concentrations of IGE.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS

Specificity

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with IgE, and have essentially no reactivity with IgG, IgM, IgA or any other mouse serum proteins.

Serum from the following species showed no significant reactivity at 1:400 dilution: mouse, rat, hamster, guinea pig, bovine, pig, horse, sheep, goat, dog, cat, rabbit or chicken; also 10% neonatal bovine serum.

Normal Range

Assay of IgE in stored sera from fourteen (14) individual Swiss mice ranged from 1 to 60 ug/ml. Each laboratory should determine expected values of its own testing population.

Precision

Samples containing low, medium and high concentrations of IgE, representing 3 different sera, were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficient of variations were calculated for the concentrations using a point-to-point curve-fitting program.

IgE concentrations were measured with good within-assay (5.2 to 8.6 %CV) and very good between-assay (2.7 to 7.3 %CV) reproducibility.

Sample	**IgE** ng/ml	**Intra-assay** %CV	**Inter-assay** %CV
Mouse A	9.7	**8.0**	**2.7**
Mouse B	44.4	**8.6**	**6.9**
Mouse C	84.6	**5.2**	**7.3**

Continued on Page 7.

CALCULATION OF RESULTS

The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, IgE concentrations may be determined as follows:

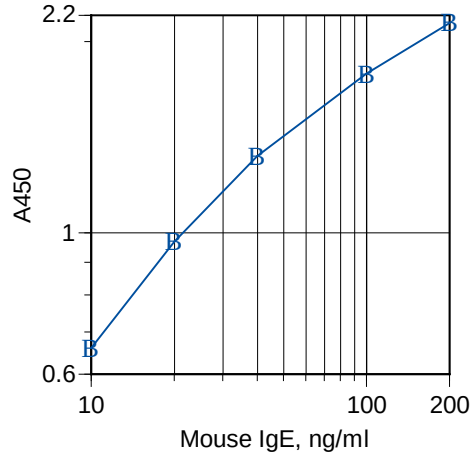
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of IgE (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The IgE concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 200 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples		A450 nm	**IgE** ng/ml
A1, A2	**Negative Diluent Control**		0.05	0
B1, B2	10 ng/ml	0.66	10	10
C1, C2	20 ng/ml	0.97	20	20
D1, D2	40 ng/ml	1.32	40	40
E1, E2	100 ng/ml	1.78	100	100
F1, F2	200 ng/ml	2.14	200	200
G1, G2	**Positive Serum Control** [Value: 92 - 140 ng/ml]		1.9	126
H1, H2	**Sample** [Diluted 1:200]		1.47	54.4
	Calculated: 200-fold dilution x 54.4 ng/ml = **10.9** ug/ml in serum			

A typical assay Standard Curve (do not use for calculating sample values)



KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at RT until kit is used entirely.
Anti-Mouse IgE - HRP Conjugate Concentrate (100x) Part No. 6374, 0.15ml	Peroxidase conjugated anti-mouse IgE in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8ºC storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Mouse IgE Microwell Strip Plate	6371	8-well strips (12)	Coated with purified anti-Mouse IgE antibodies.
Mouse IgE Standards			
10 ng/ml 20 ng/ml 40 ng/ml 100 ng/ml 200 ng/ml	6373B 6373C 6373D 6373E 6373F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing Mouse serum with calibrated IgE concentrations; diluted in buffer with protein, detergents and antimicrobiaas stabilizers.
Positive Control [IgE] range on label	6372	0.65 ml	Mouse serum with stated IgE concentration range; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 1-15ml tubes for diluting samples, anti-mouse IgE-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent Concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera are not assayed immediately , store refrigerated for up to 2 weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.
The use of plasma has not been investigated, but should be a suitable specimen for assay.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and Anti-mouse IgE-HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site for Prolcin-300 (0.1% v/v in standards, and assay buffers).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls A Positive Serum Control is provided with the kit, assigned with an IgE concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run; this blank signal should be <0.3 OD.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer is recommended. ELISA reader and pipettes should be properly calibrated.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 200-fold are appropriate for most normal mouse sera. [Example: 5ul sample + 995ul Sample Diluent]

DO NOT dilute the Standards or Positive Control Serum.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes before sample addition.
- Aspirate the liquid and pat dry on a paper towel.

2. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-mouse IgE-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

5. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.