ELISA kits available from ADI:

Catalog# **ProdDescription**

Catalog# Description
6410 Rat serum amyloid P (SAP) ELISA Kit, 96 tests, Quantitative
6410-10 Rat IgA ELISA kit 96 tests, Quantitative
6420 Rat IgG ELISA Kit, 96 tests, Quantitative

6420-RDT-25 TruStrip RDT Rat IgG Rapid Test cards, 10/pk

6430 Rat IgG1 ELISA Kit, 96 tests, Quantitative
6430-10 Rat interleulin-6 (rIL-6) ELISA Kit, high sensitivity, 96 tests, Quantitative
6430-20 Mouse interleulin-6 (rIL-6) ELISA Kit, 96 tests, Quantitative
6430-30 Human interleulin-6 (hIL-6) ELISA Kit, 96 tests, Quantitative
6440 Rat IgG2a ELISA Kit, 96 tests, Quantitative
6450 Rat IgG2b ELISA Kit, 96 tests, Quantitative
6470 Rat IgE ELISA Kit, 96 tests, Quantitative
6480 Rat IgM ELISA Kit, 96 tests, Quantitative
6490 Rat Alpha-1 Glycoprotein (A1-AGP) ELISA kit 96 tests, Quantitative
600-660-RMY Rat Myoglobin ELISA Kit

1800 Human IgE ELISA Kit, 96 tests, Quantitative
300-190-CGE Cat IgE ELISA Kit, 96 tests, Quantitative
400-190-DGE Dog IgE ELISA Kit, 96 tests, Quantitative
6470 Rat IgE ELISA Kit, 96 tests, Quantitative
7070 Monkey IgE ELISA Kit, 96 tests, Quantitative
7075 Chimp IgE ELISA Kit, 96 tests, Quantitative
7540 Goat IgE ELISA Kit, 96 tests, Quantitative
7650 Sheep IgE ELISA Kit, 96 tests, Quantitative
7750 Horse IgE ELISA Kit, 96 tests, Quantitative

Monkey: IgM, IgG, IgA, IgE
Rat: Albumin, CRP, IgG, IgM, Alpha-1- Acid glycoprotein
Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgE, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Troponin-I, TNF-alpha

Autoimmune Antibody detection kits for ANA, ssDNA, dsDNA, Histone, Sm, RNP, SSA, SSB, Scl70, Ovalbumin, Cardiolipin, CIC

Chicken: IgG, IgM, IgY, Ovalbumin **Turkey**: IgG
Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin
Pig: Albumin, IgG, IgM **Dog:** CRP, IgG, IgM
Cat: IgG, IgM **Sheep:** IgG **Goat:** IgG **Rabbit**: CRP, IgG
See Details at the web site or Contact ADI

Instruction Manual No. M-6470

Rat IgE ELISA KIT

Cat. # 6470, 96 Tests

For measurement of IgE in rat serum or plasma

For research use only (RUO), not for diagnosis, cure or prevention of the disease.

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

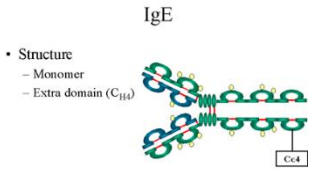
Rat IgE ELISA KIT # 6470

Kit Components, 96 tests	
Anti-rat IgE coated strip plate (8 wells x 12 strips), #6471P	1 plate
Rat IgE **Reference Standard, Lyophilized**, **Store at -20°C** , #6472	1 vial
HRP Conjugate, 11 ml, #6473	1 bottle
Sample Diluent (10X), 25 ml, #6474	1 bottle
Wash Buffer (20X), 50 ml, #6470-WB	1 bottle
TMB Substrate, 11 ml, #6470-TMB	1 bottle
Stop solution, 11 ml, #6470-SS	1 bottle
Instruction Manual, # M-6470	1

Intended Use

ADI's Rat IgE ELISA kit is a sandwich ELISA for measurement of IgE in rat serum or plasma or other biological fluids**.** This kit is for in vitro research use only (RUO).

INTRODUCTION



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.

Four subclasses of IgG are present in rat: IgG1, IgG2a, IgE and IgG2c. Respective concentrations in 80-day old Lewis rats were found to be 0.10, 0.95, 2.06 and 0.09 mg/ml (ref 1). Levels of the different subclasses vary with age and in response to immune stimulus. The rat IgG1 ELISA kit is designed for measurement of IgE in rat serum or plasma. The assay uses mouse monoclonal anti-rat IgE for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated mouse monoclonal anti-rat IgE antibodies for detection. When used as directed, the kit recognizes only IgE in rat serum. It does not recognize mouse IgG. Cross reactivity with immunoglobulins from other species has not been investigated.

Quality Control

Full set of reference standards must be run with each run. Reference standard should closely reflect the values shown in this manual. Blanks must be less than A450=0.300. Higher blanks is an indication of poor washing. Repeat the stds only with proper washing to confirm the expected values.

PERFORMANCE CHARACTERISTICS

Wash Procedure: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Expected Values: Each laboratory should establish testing ranges for the animal population being investigated.

Species Crossreactivity

This kits has not been tested with species other than rat. ADI has human, monkey, mouse, and other species IgE ELISA kits.

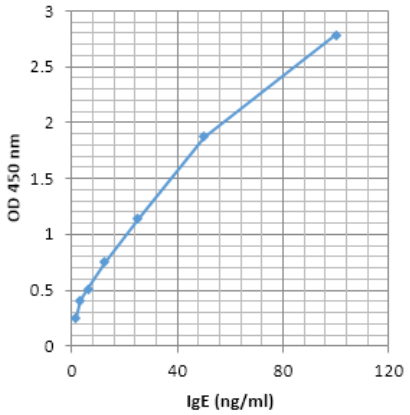
References:

1. Kinoshita M and Ross C. Quantitative analysis of immunoglobulin G subclasses in the rat.. Journal of Immunoassay. 14(3):149-166 (1993)

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples		Mean A_{450} nm	Calculated Concn
A1, A2	**Diluent**	0 ng/ml		
B1, B2	**Standard A**	1.56 ng/ml	0.25	
C1, C2	**Standard B**	3.13 ng/ml	0.41	
D1, D2	**Standard C**	6.25 ng/ml	0.51	
E1, E2	**Standard D**	12.5 ng/ml	0.75	
F1, F2	**Standard E**	25 ng/ml	1.14	
G1, G2	**Standard F**	50 ng/ml	1.87	
H1, H2	**Standard G**	100 ng/ml	2.78	

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.



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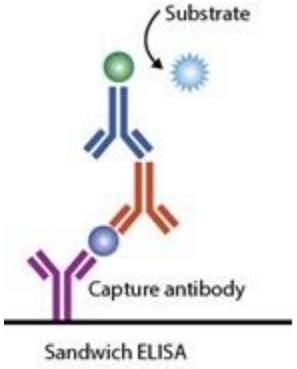
A typical assay Curve (do not use this for calculating sample values)

CALCULATION OF RESULTS:

1.Calculate the average absorbance values (A450) for blanks and each set of reference standards and samples.
2. Construct a standard curve by plotting the net mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgE in ng/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgE in the sample.
5. Ideally, PC graphing software may be used for the above steps. We find good fits of standard curve data to a one site –total and nonspecific binding model.
6. If the OD450 values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

PRINCIPLE OF THE TEST



Rat IgE ELISA kit is based on binding of Rat IgE from samples to two antibodies, one immobilized on the microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of IgE present in the sample. Adding stopping solution terminates the reaction. 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 (5-1000 ul) and multichannel pipet with disposable plastic tips. Plate shaker or orbital shaker; Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute 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. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

SPECIMEN COLLECTION and HANDLING

Collect blood by venipuncture; allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot be immediately assayed, store frozen for up to six months. Avoid repeated freezing and thawing of samples. **Cell or tissues extract samples have not been optimized.**

Sample Dilution

IgE is typically present in rat serum or plasma have large variations. In order to obtain values within range of the standard curve, we suggest that samples initially be diluted **at least 1:20-1:200** using the following procedure for each sample to be tested:

1. Prepare 1:10 dilution (5 ul sample into 45 ul 1x sample diluent or normal saline). Dilution

2. Prepare 1:100 dilution (20 ul of 1:10 sample into 180 ul 1x sample diluent or normal saline). Dilution =1:100.

   Repeat this procedure for each sample to be tested. In order to avoid matrix effects, serum dilutions less than 10-fold should be avoided. Tissue extracts and body fluids other than serum or plasma will likely have lower IgE levels than those found in serum. Optimal dilutions of such samples should be determined empirically.

REAGENT PREPARATION

1. **Dilute Wash Buffer (20x stock)**. Dilute the entire 50 ml with 950 ml of distilled or deionized water (total volume 1000 ml). Store at room temperature for the entire use of the kit. It can be stored at 4oC for long term storage.

2. **Sample Diluent** is 10X. **Dilute 1:10** with water (1 ml stock in 9 ml water). Store 1x sample diluent at 4oC..

3. **Reference Standard** is provided as lyophilized power. The rat IgE standard is provided as a lyophilized stock. *Reconstitute with 1 ml of distilled or deionized water (the reconstituted standard is stable at 4oC for one day but should be aliquoted and frozen at* ***-20°C*** *after reconstitution if future use is intended)*.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8oC until the expiration date printed on the label. The Rat IgE reference standard should be stored at -20oC.

Preparation of Standards:

1.The rat IgE standard is provided as a **lyophilized stock**. **Reconstitute with 0.1 ml of** distilled or deionized water (the reconstituted standard is stable at 4oC for one day but should be aliquoted and frozen at -20oC after reconstitution if future use is intended).

1. Label 8 polypropylene or glass tubes as 100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0 ng/ml.
2. Into the tube labeled 100 ng/ml, pipette the volume of diluent detailed on the IgE standard vial label. Then add the indicated volume of IgE standard (shown on the IgE standard vial label) and mix gently. This provides the 100 ng/ml standard.
3. Dispense 250 ul of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.13, 1.56, and 0 ng/ml.
4. Prepare a 50 ng/ml standard by diluting and mixing 250 ul of the 100 ng/ml standard with 250 ul of diluent in the tube labeled 50 ng/ml.
5. Similarly prepare the 25, 12.5, 6.25, 3.13, and 1.56 ng/ml standards by 2-fold serial dilution.

Notes: When preparing the serial dilutions of the standards gently mix the standards for 5-10 seconds and then take aliquots to make further dilutions. Following the above dilution scheme.

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

Label or mark the microtiter well strips to be used on the plate.

1. Use first 2 wells for blanks (100 ul of 1x sample diluent). Pipet **100 ul standards and samples** in duplicate into appropriate wells. Mix gently, and incubate at room temperature (25°C) for **45 minutes on an orbital shaker (100-150 rpm).** If an automated shaker is not available, the plate can be mixed manually every few minutes.

2. Remove or aspirate the plate contents and **wash the wells 5 times** with 400 ul of 1x wash buffer using an automated washer. If washing manually then dump the plate contents and tap over paper towels, add wash buffer, shake the contents of 5-10 seconds and repeat the steps. Tap the plate over fresh paper towels between each washing.

3. Pipette **100 ul of HRP conjugate** into each well, and incubate at room temperature (25°C) for **45 minutes on an orbital shaker (100-150 rpm).**

4. Remove or aspirate the plate contents and **wash the wells 5-6 times** with 400 ul of 1x wash buffer as above in step 5.

5. **Add 100 ul of TMB Substrate** into each well. Mix gently. Cover the plate and incubate for **20 minutes** at 25°C **on an orbital shaker (100-150 rpm)**. **Blue color develops in standards and positive wells**. This step can be reduced or increased by ± 5 minutes to keep the color within reading range. If your ELISA reader cannot read above A450 of 2.00-3.00 then reduce the incubation time.

6. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 30 seconds. **Blue color turns yellow.**

7. Measure the **absorbance at 450 nm** using an ELISA reader. Color is stable for at least 30 minutes after stopping.

Please Note: Due to plate reader differences, the high standard absorbance values may be out of range occasionally. If this occurs, absorbance values may be determined at 405 nm instead. If absorbance values exceed the high standard, the samples should be appropriately diluted and redetermined. Samples with absorbance values below those of the lowest standard should be assigned a zero IgA value.