Sample Recovery

High and low concentrations of rat IgG were mixed into each of 3 serum samples. Observed assay values compared to expected values ranged from 97 to 114%, indicating accurate quantification of IgG in rat serum.

Sample	Expected ng/ml	Observed ng/ml	Observed/ Expected
High IgG Concn		97	
+ Rat serum C, 73 ng/ml	170	178	**105**
+ Rat serum D, 111 ng/ml	207	218	**105**
+ Rat serum F, 66 ng/ml	162	157	**97**
Low IgG Concn		23	
+ Rat serum C, 73 ng/ml	96	109	**114**
+ Rat serum D, 111 ng/ml	134	142	**106**
+ Rat serum F, 66 ng/ml	89	87	**98**

Related Items

Catalog# **Product 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
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

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Instruction Manual No. M-6420

Rat IgG ELISA Kit

Cat. No. 6420, 96 Tests

For Quantitative Determination of Rat IgG
Serum, plasma or or other biological fluids

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

ALPHA DIAGNOSTIC
INTERNATIONAL

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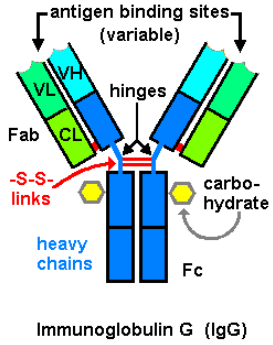
Web Site: www.4adi.com

INTENDED USE

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

RESEARCH USE OF THE TEST

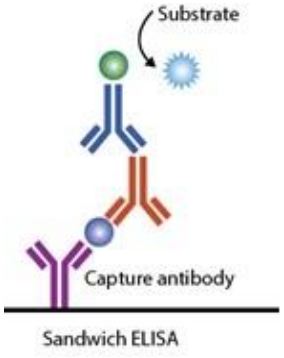


Immunoglobulin G (IgG)

Immunoglobulin G (IgG) is a type of antibody. It is a protein complex composed of four peptide chains—two identical heavy chains and two identical light chains arranged in a Y-shape typical of antibody monomers. IgG has molecular weight of approximately 150 kDa, heavy or H chain approximately 50 kDa and light or L chain 25 kDa. Each IgG has two antigen binding sites. Representing approximately 75% of serum antibodies in humans, IgG is the most common type of antibody found in the circulation. Levels of total IgG, IgA and/or IgM can reveal health status or results of experimental or pathological conditions (e.g., hypo- or hyper- gammaglobulinemia or acute or chronic infection). Also, measurements of specific antibody levels, in antigen-specific assays, are often best interpreted relative to values of total IgG, IgA, and IgM in the sample and/or individual.

Immunoassays using heavy-chain specific antibodies provide for selective, sensitive quantification of rat immunoglobulins IgG, IgA and IgM, as found circulating in blood or as present in other body fluids, including saliva, milk/colostrum, ascites, tears and mucosa of linings of the gut, respiratory or urigenital tracts. The quantitative immunoassay measures rat IgG with high sensitivity; this allows dilution beyond interference from the sample matrix for samples derived from any of the above specimen types. Expected performance relative to precision, recovery and linearity of dilution is presented for guidance of use and experimental design.

PRINCIPLE OF THE TEST



The Rat IgG ELISA kit is based on the binding of rat IgG 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 IgG 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 IgG in samples and control is calculated from a curve of standards containing known concentrations of IgG.

PERFORMANCE CHARACTERISTICS & EXPECTED RESULTS

Specificity

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

Serum from the following species showed no significant reactivity at 1:500 dilution: human, monkey, mouse, hamster, guinea pig, bovine, pig, horse, sheep, goat, dog, cat, rabbit or chicken; also 10% neonatal bovine serum.

Normal Range

Assay of IgG in stored sera from twenty (20) individual adult rats ranged from 7.9 to 24 mg/ml (median = 13.6 mg/ml). Each laboratory should determine expected values of its own testing population.

Precision

Samples containing low, medium and high concentrations of IgG 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 (CVs) were calculated for the concentrations using a point-to-point curve-fitting program.

IgG concentrations were measured with good within-assay (2.6 to 7.0 %CV) and between-assay (5.2 to 8.8 %CV) reproducibility.

Sample	IgG ng/ml	Intra-assay %CV	Inter-assay %CV
Low Sample	27	2.6	6.3
Mid Sample	66	7.0	8.8
High Sample	110	3.3	5.2

Linearity of Dilution

Three (3) individual stored sera and purified rat IgG were diluted to 2 levels for testing, and concordance of the assay values was compared. Agreement of values ranged from 95 to 100%, demonstrating linear dilution and equivalent quantification across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Rat Serum 1	1:100k 1:800k	168 23	16.8 18.4	**95 %**
Rat Serum 2	1:100k 1:800k	210 24	21.0 19.2	**96 %**
Rat Serum 3	1:50k 1:400k	129 15.5	6.45 6.20	**98 %**
Rat IgG	1:10 1:40	279 69	2.79 2.76	**100 %**

CALCULATIONS

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

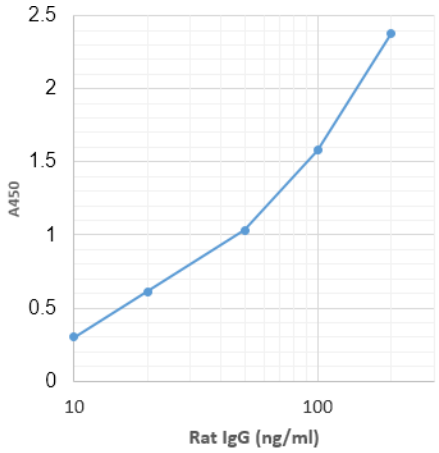
1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of Rat IgG (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The Rat IgG 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	Rat IgG ng/ml
1A, B	**Negative Diluent Control**	0.06	0
1C, D	10 ng/ml **Standard**	0.30	10
2E, F	25 ng/ml **Standard**	0.61	20
3G, H	50 ng/ml **Standard**	1.03	50
2A, B	100 ng/ml **Standard**	1.58	100
2C, D	200 ng/ml **Standard**	2.38	200
2E, F	**Positive Serum Control** [Value: 56 - 104 ng/ml]	1.40	84
2G, H	**Sample** [Diluted 1:100k]	1.69	113
	Calculated: 100k-fold dilution x 113 ng/ml = **11.3** mg/ml in serum		

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



KIT CONTENTS

Ready For Use: Store as indicated on labels.

Component	Part #	Amt	Contents
Anti-Rat IgG Microwell Strip Plate	6421	8-well strips (12)	Coated with purified anti- Rat IgG antibodies. Return unused strips to the pouch with desiccant; re-seal and store refrigerated.
Positive Control [IgG] range on label	6422	0.65 ml	Rat serum with stated IgG concentration range; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
Rat IgG Standards			
10 ng/ml	6423B	0.65 ml	Five (5) vials, each containing rat serum calibrated using purified rat IgG; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
25 ng/ml	6423C	0.65 ml	
50 ng/ml	6423D	0.65 ml	
100 ng/ml	6423E	0.65 ml	
200 ng/ml	6423F	0.65 ml	
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

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, to 1L with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-Rat IgG-HRP Conjugate Concentrate (100x) Part No. 6424, 0.15ml	Peroxidase conjugated anti-rat IgG antibody 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.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipetter is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody-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.

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 kit box label. Stabilities of the working solutions are indicated under Reagent Preparation.

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

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference.

For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For **other samples**, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately , store refrigerated for up to a week, or frozen for long-term storage. Avoid freeze-thaw cycles.

QUALITY CONTROL

Sample Controls A Positive Serum Control is provided with the kit, assigned with an IgG 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; OD should be <0.3.

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^{o} C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 1:100k-1:200k are appropriate for most normal rat sera. For accuracy, three dilution steps are recommended, as follows:

1) 10ul serum + 990ul diluent = [1:100],
2) 10ul [1:100] + 990ul diluent = [1:10k],
3) 50ul [1:10k] + 950ul diluent = **[1:200k].**

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, to include 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 about 5 minutes before sample addition.
- Aspirate or dump the liquid and pat the plate 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 Working Anti-Rat IgG-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.