ELISA kits available from ADI:

Catalog#	ProdDescription
9020	Pig IgG ELISA Kit, 96 tests, Quantitative (swine/porcine)
9080	Pig IgM ELISA Kit, 96 tests, Quantitative
9000	Pig Albumin ELISA Kit, 96 tests, Quantitative
1780	Human IgA ELISA Kit, 96 tests, Quantitative
6310	Mouse IgA ELISA Kit, 96 tests, Quantitative
6440	Rat IgA ELISA Kit, 96 tests, Quantitative
6510	Rabbit IgA ELISA Kit, 96 tests, Quantitative
7010	Monkey IgA ELISA Kit, 96 tests, Quantitative
7720	Horse IgA ELISA Kit, 96 tests, Quantitative
8075	Bovine IgA ELISA Kit, 96 tests, Quantitative
600-610-HMY	Human Myoglobin ELISA Kit
600-620-MMY	Monkey Myoglobin ELISA Kit
600-630-MMY	Mouse Myoglobin ELISA Kit
600-650-RMY	Rabbit Myoglobin ELISA Kit
600-660-RMY	Rat Myoglobin ELISA Kit

Human: 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, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, estradiol, testosterone, progesterone).

Monkey: IgM, IgG, IgA, IgE
Rat: Albumin, CRP, IgG, IgM, Alpha-1- Acid glycoprotein
Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, 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-6450

Rat IgG2b ELISA KIT

Cat. # 6450, 96 Tests

For measurement of IgG2b in rat serum or plasma

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

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 West • San Antonio• Texas 78249 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777

Email: Techsupport@4adi.com
Web Site: www.4adi.com

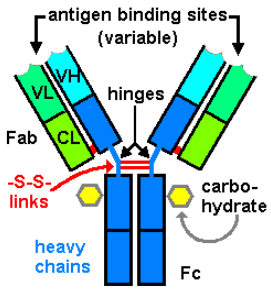
Rat IgG2b ELISA KIT # 6450

Kit Components, 96 tests	Cat #
Anti-rat IgG2b **coated strip plate** (8 wells x 12 strips), #6451P	1 plate
Rat IgG2b **Reference Standard**, **Lyophilized**, **Store at -20°C** , #6452	1 vial
HRP Conjugate, 11 ml, #6453	1 bottle
Sample Diluent (10X), 25 ml, #6454	1 bottle
Wash Buffer (20X), 50 ml, #6450-WB	1 bottle
TMB Substrate, 11 ml, #6450-TMB	1 bottle
Stop solution, 11 ml, #6450-SS	1 bottle
Instruction Manual, # M-6450	1

Intended Use

ADI's Rat IgG2b ELISA kit is a sandwich ELISA for measurement of IgG2b in rat serum or plasma or other biological fluids**.** This kit is for in vitro research use only (RUO).

INTRODUCTION



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.

Four subclasses of IgG are present in rat: IgG1, IgG2a, IgG2b 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 IgG2b in rat serum or plasma. The assay uses mouse monoclonal anti-rat IgG2b for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated mouse monoclonal anti-rat IgG2b antibodies for detection. When used as directed, the kit recognizes only IgG2b 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.

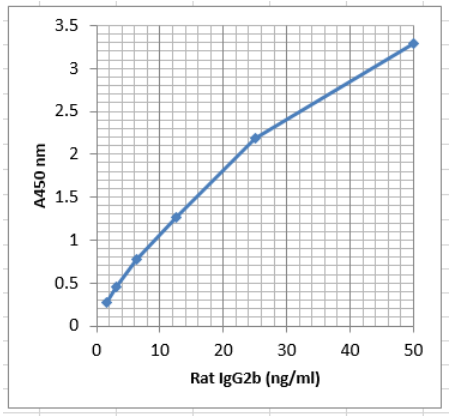
References:

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	0.266	
C1, C2	**Standard B**	3.13 ng/ml	0.458	
D1, D2	**Standard C**	6.25 ng/ml	0.772	
E1, E2	**Standard D**	12.5 ng/ml	1.271	
F1, F2	**Standard E**	25 ng/ml	2.187	
G1, G2	**Standard F**	50 ng/ml	3.289	

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.



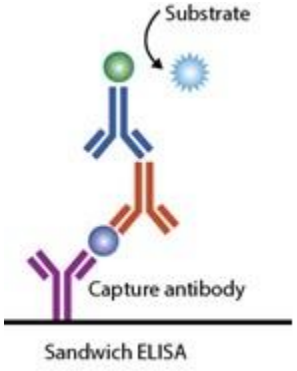
ADI_ELISA4

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 IgG2b in ng/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgG2b 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 IgG2b ELISA kit is based on binding of Rat IgG2b 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 IgG2b 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 IgG2b 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

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).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

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

IgG2b is typically present in rat serum or plasma at concentrations of 0.5- 2 mg/ml. In order to obtain values within range of the standard curve, we suggest that samples initially be diluted **50,000** fold using the following procedure for each sample to be tested:

1. Prepare 1:100 dilution (5 ul sample into 495 ul 1x sample diluent or normal saline). Dilution
2. Prepare 1:10,000 dilution (5 ul of 1:100 sample into 495 ul 1x sample diluent or normal saline). Dilution =1:10,000.
3. Prepare 1:50,000 dilution (50 ul of 1:10,000 sample into 200 ul 1x sample diluent). Dilution =1:50,000.

   Repeat this procedure for each sample to be tested. In order to avoid matrix effects, serum dilutions less than 10,000 fold should be avoided. Tissue extracts and body fluids other than serum or plasma will likely have lower IgG2b 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 IgG2b 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 IgG2b reference standard should be stored at -20oC.

Preparation of Standards:

1.The rat IgG2b standard is provided as a **lyophilized stock**. **Reconstitute with 1.0 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).

2. Label 6 polypropylene or glass tubes as 50, 25, 12.5, 6.25, 3.13 and 1.58 ng/ml.

3. Into the tube labeled 50 ng/ml, pipette the volume of diluent detailed on the IgG2b standard vial label. Then add the indicated volume of IgG2a standard and mix gently. This provides the 50 ng/ml standard.

4.Dispense 250 µl of diluent into the tubes labeled 25, 12.5, 6.25, 3.13, 1.58 and 0 ng/ml.

5. Prepare a 25 ng/ml standard by diluting and mixing 250 µl of the 50 ng/ml standard with 250 µl of diluent in the tube labeled 25 ng/ml.

6. Similarly prepare the 12.5, 6.25, 3.13 and 1.58 ng/ml standards by 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.