

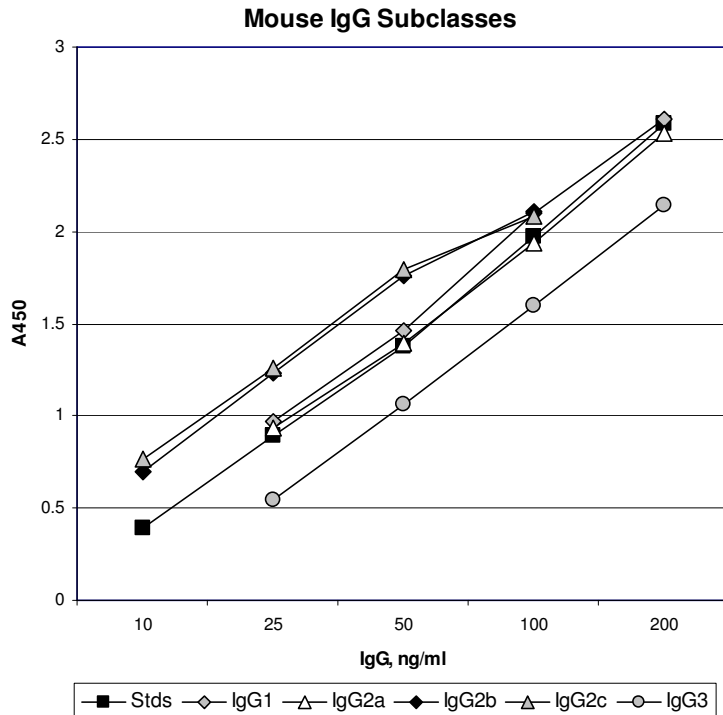
PERFORMANCE CHARACTERISTICS (continued)

IgG Subclass Quantitation

The anti-mouse IgG capture and detection antibodies have been selected for balanced recognition of all subclasses of mouse IgG, including IgG3 and IgG2c (which is the IgGa analog in mouse strains C57Bl/6, C57Bl/10, SJL, NOD). While this provides for most accurate quantitation of polyclonal IgG in serum and other biological fluids of all mouse strains, the assay is also suitable for accurate quantitation of monoclonal antibodies where significant idiotypic (individual) and allotypic (strain) reactivity differences often produce non-parallelism with the standard curve and under-reporting of certain subclasses (especially IgG3 and IgG2c) in IgG assays with non-balanced subclass recognition.

The following figure depicts dilutions of monoclonal antibodies of different IgG subclasses with concentrations assigned from A280 values of purified preparations.

All dilution curves were essentially parallel to the Standard Curve, which contains total polyclonal IgG and is prepared from a pool of varied strains of normal adult mice.



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

Instruction Manual No. M-6320

Mouse IgG

ELISA Kit Cat. No. 6320

**For Quantitative Determination of Mouse
Immunoglobulin G in Fluids**



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INTENDED USE

The Mouse IgG ELISA Kit is an in vitro immunoassay for the quantification of IgG circulating in serum or in other appropriately qualified samples from tissue fluids (e.g., saliva, mucosa), or in cultures of mouse cells including hybridomas.

RESEARCH USE OF THE TEST

Immunoassays using heavy-chain specific antibodies provide for selective, sensitive quantification of mouse 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 urogenital tracts.

Levels of total IgG, IgA and/or IgM can reveal health status or results of experimental or pathological conditions (e.g., hypo- or hypergammaglobulinemia 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.

The assays are also suitable for quantifying monoclonal antibodies and can be a reliable method for monitoring production and establishing quality control.

The quantitative immunoassays measure mouse IgG, IgA and IgM with high sensitivity; this allows dilution beyond interference from the sample matrix for samples derived from any of the above specimen types. Also, each assay is Ig class specific, such that all IgG or IgA subclasses are reliably quantified in essentially any specimen, freshly obtained and/or suitable stored. Expected performance of each kit relative to precision, recovery and linearity of dilution is presented as guidance for use and experimental design.

PRINCIPLE OF THE TEST

The Mouse IgG ELISA kit is based on the binding of mouse 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.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the 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 all IgG subclasses: IgG1, IgG2a, IgG2b, IgG2c and IgG3; and have essentially no reactivity with IgM, IgA, IgE 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 IgG in stored sera from fourteen (14) individual Swiss mice ranged from 1 to 19 mg/ml (median = 3.1mg/ml). Total IgG varies with sex, age and strain. 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 were calculated for the concentrations using a point-to-point curve-fitting program.

IgG concentrations were measured with good within-assay (3.5 to 6.7 %CV) and between-assay (2.8 to 7.2 %CV) reproducibility.

Sample	IgG ng/ml	Intra-assay %CV	Inter-assay %CV
Low Level	34.1	5.2	2.8
Medium Level	77.6	3.5	3.0
High Level	103.7	6.7	7.2

Linearity of Dilution

Three (3) individual stored sera and purified IgG were diluted to 2 levels for testing, and concordance of the assay values were compared. The mean recovery ranged from 92 to 100%, demonstrating linear dilution and equivalent quantification across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Mouse M1	1:40k	178	7.13	92 %
	1:160k	38	6.13	
Mouse F1	1:40k	234	9.35	100 %
	1:320k	38	9.34	
Mouse M2	1:20k	177	3.53	95 %
	1:80k	49	3.90	
Mouse IgG	1:6.25k	144	0.90	96 %
	1:50k	16.6	0.83	

CALCULATION OF RESULTS

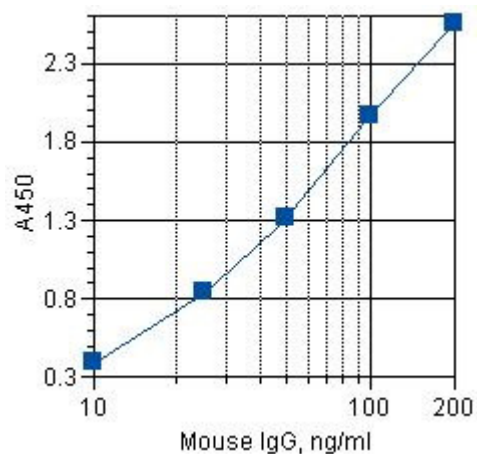
- The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, IgG concentrations may be determined as follows:
- Calculate the mean OD of duplicate samples.
- On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of 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.
- The IgG concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
- Multiply the values obtained for the samples by the dilution factor of each sample.
- 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	IgG ng/ml
A1, A2	Negative Diluent Control	0.09	0
B1, B2	10 ng/ml Standard	0.39	10
C1, C2	25 ng/ml Standard	0.84	25
D1, D2	50 ng/ml Standard	1.32	50
E1, E2	100 ng/ml Standard	1.96	100
F1, F2	200 ng/ml Standard	2.56	200
G1, G2	Positive Serum Control [Value: 56 - 104 ng/ml]	1.68	79
H1, H2	Sample [Diluted 1:160k] Calculated: 160k-fold dilution x 33.5 ng/ml = 5.36 mg/ml in serum	1.01	33.5

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 2-8°C 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 IgG - HRP Conjugate Concentrate (100x) Part No. 6324, 0.15ml	Peroxidase conjugated anti-mouse IgG in buffer with protein, detergents and ProClin 300 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 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Mouse IgG Microwell Strip Plate	6321	8-well strips (12)	Coated with purified anti-Mouse IgG antibodies.
Mouse IgG Standards			
10 ng/ml	6323B	0.65 ml	Five (5) vials, each containing mouse serum with calibrated IgG concentrations; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
25 ng/ml	6323C	0.65 ml	
50 ng/ml	6323D	0.65 ml	
100 ng/ml	6323E	0.65 ml	
200 ng/ml	6323F	0.65 ml	
Positive Control [IgG] range on label	6322	0.65 ml	Mouse serum with stated IgG concentration range; diluted in buffer with protein, detergents and ProClin 300 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 5-15ml tubes for diluting samples and Anti-mouse IgG-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 IgG-HRP contain Proclin 300 (0.05%, v/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.

MSDS for TMB, sulfuric acid and Proclin 300, if not already on file, can be requested or obtained from the ADI website.

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 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.

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.

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 40 to 100k-fold are appropriate for most normal mouse sera. For accuracy, three dilution steps are recommended, as follows:

- 1) 10ul serum + 390ul diluent = [1:40],
- 2) 20ul [1:40] + 980ul diluent = [1:2k],
- 3) 20ul [1:2k] + 980ul diluent = **1:100k**

DO NOT dilute the Standards or Control.

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 - 60min; 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 is recommended. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation

[100ul - 30min; 5 washes]

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

4. Substrate Incubation

[100ul - 15min]

- 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, assuring the top standard does not surpass 2 OD.

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.