LSBio™ Mouse IgG ELISA Kit

Catalog No. LS-F10451

User Manual

Please Read the Manual Carefully
Before Starting your Experiment

LSBio
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Mouse IgG Antigen ELISA Kit

Strip well format. Reagents for up to 96 tests.

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INTENDED USE

This Mouse Immunoglobulin G (IgG) antigen assay is intended for the quantitative determination of total mouse IgG antigen in serum, plasma, hybridoma cell supernatants, ascites or other biological fluids. This assay does not distinguish IgG subclasses. **For research use only.**

BACKGROUND

IgG is the most abundant immunoglobulin in serum and is predominately involved in the secondary immune response. The IgG subclasses are designated 1, 2, 3 and 4 based on their relative prevalence in human serum.

ASSAY PRINCIPLE

Mouse IgG will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, horseradish peroxidase labeled polyclonal anti-mouse IgG antibody binds to the captured protein. Excess antibody is washed away and TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of mouse IgG. Color development is directly proportional to the concentration of total IgG in the samples.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-mouse IgG antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **5X Diluent:** 1 bottle of 50ml
- **Mouse IgG standard:** 1 vial lyophilized standard
- **Anti-mouse horseradish peroxidase antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard may be stored at -80°C for later use. Do not freeze-thaw the standard more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard• Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **1X Diluent:** 5X Diluent may contain precipitate. Warm to redissolve before use. Dilute 50ml of 5X diluent concentrate with 200ml of deionized water.
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water.

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of diluent directly to the vial and agitate gently to completely dissolve contents. This will result in a 1,000ng/ml standard solution.

Dilution table for preparation of mouse IgG standard:

IgG concentration (ng/ml)	Dilutions
500	500µl Diluent + 500µl (from std vial)
250	500µl Diluent + 500µl (500ng/ml)
100	600µl Diluent + 400µl (250ng/ml)
50	500µl Diluent + 500µl (100ng/ml)
25	500µl Diluent + 500µl (50ng/ml)
10	600µl Diluent + 400µl (25ng/ml)
5	500µl Diluent + 500µl (10ng/ml)
2.5	500µl Diluent + 500µl (5ng/ml)
1	600µl Diluent + 400µl (2.5ng/ml)
0.5	500µl Diluent + 500µl (1ng/ml)
0	500µl Diluent Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl IgG standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures IgG antigen in the 0.5-500 ng/ml range. If the unknown is thought to have high IgG levels, dilutions may be made in diluent. A

1:1,000,000 dilution for normal mouse plasma is suggested for best results.

Antibody Addition

Briefly centrifuge vial before opening. Dilute 2µl of conjugated antibody in 10ml of diluent and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

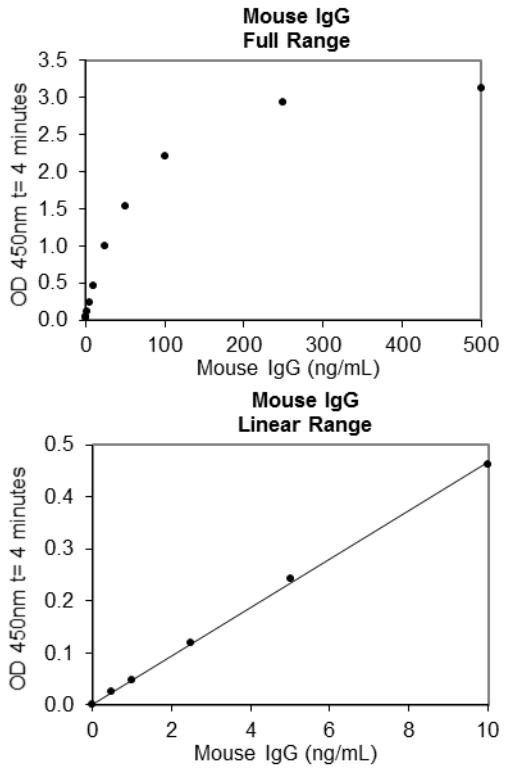
Measurement

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of IgG in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of IgG in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):



EXPECTED VALUES

The concentration of IgG in normal mouse serum ranges from 5 to 12 mg/mL.

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD450: 0.05-0.059) and calculating the corresponding concentration. The MDD was 0.119 ng/ml.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	1.84	15.9	141
Standard Deviation	0.116	0.700	12.8
CV (%)	6.29	4.41	9.10

Inter-assay Precision: Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2	3
n	10	10	10
Mean (ng/ml)	2.04	20.5	127
Standard Deviation	0.143	0.936	12.9
CV (%)	7.01	4.56	10.2

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	1.97	7.44	32.4	147
Average % Recovery	98	99	93	98
Range	91-109%	94-102%	88-100%	86-109%

Linearity: To assess the linearity of the assay, pooled citrated mouse plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	99	103	99	98
Range	96-104%	96-107%	89-109%	95-101%

Specificity: This assay recognizes total mouse IgG. Pooled normal plasma from rat, rabbit, sheep, pig, and human were assayed, and no significant cross reactivity was observed.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (mg/mL)
Citrate Plasma	1:100,000	10.3
	1:250,000	11.2
	1:500,000	10.5
	1:1,000,000	8.68
	1:2,000,000	10.9
	1:4,000,000	8.89

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.5 ng/ml	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	500 ng/ml	
B	0	0.5 ng/ml	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	500 ng/ml	
C												
D												
E F												
G												
H												

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion.

Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

Returns, Refunds, Cancelations: Any problems with LifeSpan products must be reported to LifeSpan within 10 days of product receipt. The customer must obtain written authorization from LifeSpan before returning items. To request that goods be returned, please contact LifeSpan Technical Support. If an error by LifeSpan BioSciences results in shipment of an incorrect order, LifeSpan will, at its option, either ship a replacement order at no charge, or credit the customer's account for the original product shipped in error. Returns and cancelations may be subject to a 30% restocking fee.

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