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Mouse IgG ELISA Kit

Strip well format. Reagents for up to 96 tests

Catalog No.	**CS234A** **CS234B**	**Quantity:**	**1 x 96 tests** **5 x 96 tests**

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.

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 proportional to the concentration of IgG in the samples.

Reagents Provided:

♦ **96 well microtiter strip plate**
8X12 removable well strips containing affinity purified anti-mouse IgG antibody coated on the blocked and dried surface.
♦ **10X Wash Buffer:**
1 bottle of 50ml; bring to 1X using DI water
♦ **Mouse IgG antigen standard:**
1 vial of lyophilized standard
♦ **Peroxidase anti-mouse IgG antibody:**
1 vial of lyophilized HRP labeled antibody
♦ ¨**TMB substrate solution:**
1 bottle of 10ml solution

Storage and Stability: All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

Reagents and Equipment Required:

•1-channel pipettes covering 0-10 µl and 200-1000 µl
•12-channel pipette covering 30-300µl
•Paper towels or kimwipes
•50 ml tubes, 1.5 ml centrifuge tubes
•1N H_2SO_4
•DI water

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•Magnetic stirrer and stir-bars
•Plastic containers with lids
•Microtiter plate spectrophotometer operable at 450 nm
•Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm.

Warnings: **Warning** – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

Precautions:
- **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.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

Preparation of Reagents:
- TBS: 0.1M Tris 0.15M NaCl, pH 7.4
- Blocking buffer (BB): 3% BSA in TBS
- Wash buffer concentrate: The wash buffer is supplied in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.

Specimen Collection: The assay measures total mouse IgG in the 0.1-100ng/mL range. Samples giving mouse IgG levels above 100ng/mL should be diluted in BSA blocking buffer before use. A 1:1,000,000 to 1:20,000,000 dilution for serum is suggested for best results. This dilution can be generated by three 1:100 serial dilutions and one 1:20 dilution.

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

Preparation of Standard:
Reconstitute standard vial with 1 mL of blocking buffer (BB) to give a 1,000ng/mL solution.

Dilution table for preparation of mouse IgG standards:

IgG concentration (ng/mL)	Dilutions
100	900µL BB + 100µL (1,000ng/mL vial)
50	500µL BB + 500µL (100ng/mL)
25	500µL BB + 500µL (50ng/mL)
10	600µL BB + 400µL (20ng/mL)
5	500µL BB + 500µL (10ng/mL)
2.5	500µL BB + 500µL (5ng/mL)
1	600µL BB + 400µL (2ng/mL)
0.5	500µL BB + 500µL (1ng/mL)
0.25	500µL BB + 500µL (0.5ng/mL)
0.1	600µL BB + 400µL (0.2ng/mL)
0	500µL BB 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. Add 100µL 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.

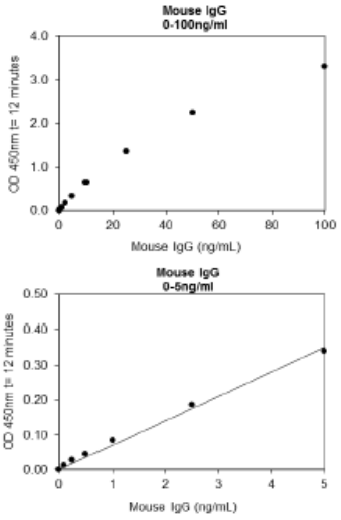
Peroxidase Antibody Addition:
Reconstitute peroxidase conjugated antibody by adding 10mL blocking buffer to vial. Agitate gently to completely dissolve contents. Add 100µL to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µL wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation:
Add 100µL TMB substrate to all wells and shake plate for 5-15 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µL of 1N H_2SO_4 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 and read final absorbance values at 450nm. For best results read plate immediately.

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}).

Assay Calibration:
Plot A_{450} against the amount of Mouse IgG in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total Mouse IgG in the unknowns can be determined from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)



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Expected Values: The concentration of IgG in normal mouse serum ranges from 5 to 12 mg/mL. The kit does not cross react significantly with rat, human, guinea pig, or rabbit IgG.

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 or from contact with the above product.

Example of Kit Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1ng/mL	0.2ng/mL	0.5ng/mL	1ng/mL	2ng/mL	5ng/mL	10ng/mL	20ng/mL	50ng/mL	100ng/mL	
B	0	0.1ng/mL	0.2ng/mL	0.5ng/mL	1ng/mL	2ng/mL	5ng/mL	10ng/mL	20ng/mL	50ng/mL	100ng/mL	
C												
D												
E												
F												
G												
H												

96 Well Plate
Standards: 22 wells
Samples: 74 wells

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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