Mouse IgG ELISA Kit

Catalog No.: IMSIGGKT **Lot No.:** 512

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

Innovative Research, Inc
www.innov-research.com | support.innov-research.com
Ph: 248.896.0145 | Fx: 248.896.0149

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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 ar dried surface.

10X Wash Buffer:

1 bottle of 50mL; bring to 1X using DI water

5X Diluent:

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 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 0-10µL and 200-1000µL

•12-channel pipette covering 30-300µL

•Paper towels or kimwipes

•50mL tubes, 1.5mL centrifuge tubes

•1N H_2SO_4

•DI water

•Magnetic stirrer and stir-bars

•Plastic containers with lids

•Microtiter plate spectrophotometer operable at 450nm

•Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

Caution and Warnings

Warning – Avoid skin and eye contact when using TMB substrate solution. It may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

Sample Collection

The assay measures total mouse IgG in the 1-1000ng/mL range. Samples giving mouse IgG levels above 1000ng/mL should be diluted in BSA blocking buffer before use. A 1:1,000,000 dilution for normal plasma is suggested. This dilution can be generated by three 1:100 serial dilutions. Optimal dilutions should be experimentally determined by the researcher

Reagent Preparation

Diluent concentrate: The diluent is supplied in a 5X concentrate and must be diluted 1:5 with deionized water for use with the kit.

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.

Assay Procedure

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

Preparation of Standard:

Reconstitute standard vial with 1mL of 1X Diluent to give a 1,000ng/mL solution.

Dilution table for preparation of mouse IgG standards:

IgG Concentration (ng/ml)	Dilutions
1000	From reconstituted standard vial
500	500µL 1X Diluent + 500µL (1000ng/mL)
250	500µL 1X Diluent + 500µL (500ng/mL)
100	600µL 1X Diluent + 400µL (250ng/mL)
50	500µL 1X Diluent + 500µL (100ng/mL)
25	500µL 1X Diluent + 500µL (50ng/mL)
10	600µL 1X Diluent + 400µL (25ng/mL)
5	500µL 1X Diluent + 500µL (10ng/mL)
2.5	500µL 1X Diluent + 500µL (5ng/mL)
1	600µL 1X Diluent + 400µL (2.5ng/mL)
0	500µL 1X Diluent Zero point to determine background

Assay Procedure Continued

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:

Dilute 1µL into 10mL of 1X Diluent. 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:

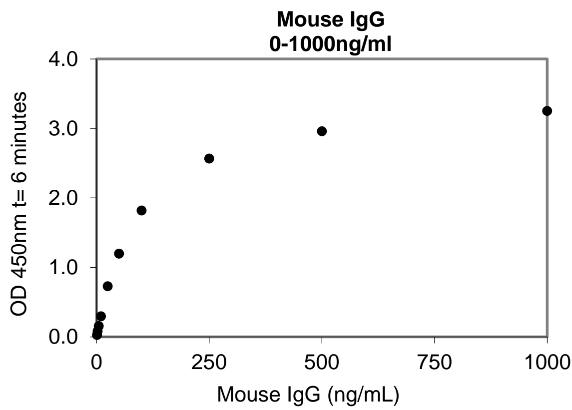
Plot A_{450} against the amount of Mouse IgG in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total Mouse IgG in the unknowns can be determined from this curve.

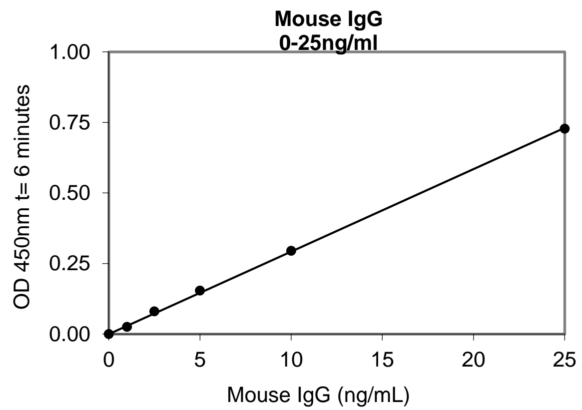
Expected Values

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.

Standard Curve Examples

Drag and drop the curve examples if in word format.





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