Catalog #80644

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Crystal Chem
High Performance Assays

Mouse IgG ELISA Kit Instructions

For the quantitative determination of IgG in mouse serum and plasma

Catalog #80644
96 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The Mouse IgG ELISA kit is for the quantitative determination of IgG in mouse serum and plasma. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures. For in Vitro Use Only.

B. Introduction

Immunoglobulin G (IgG) is the main antibody found in blood and consists of four peptide chains (two heavy and two light). Mouse IgG comes in four subtypes (IgG_1, IgG_{2a}, IgG_{2b}, and IgG_3) that each have slightly different functions and structures. Via different immune mechanisms, all bind to bacteria and viruses to protect the body and fight disease and infection. A deficiency in one, or more, subclass affects the immune response of other immunoglobulins, and understanding these mechanisms is currently under investigation.

C. Principle of the Assay

The Mouse IgG ELISA kit is a double antibody sandwich ELISA. In the assay, the IgG present in the sample binds with anti-IgG antibodies adsorbed to the surface of the microplate. In the following step, the specific anti-IgG-Antibody binds, in turn, to the immobilized IgG. The second antibody is conjugated with HRP. In the closing substrate reaction, the IgG levels of the samples can be measured by color intensity.

D. Kit Storage

1. Upon receipt of the Mouse IgG ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 CONTENTS OF KIT

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
CAL	Mouse IgG Calibrator	1 x 1 vial
DIL	Diluent (5X Concentrate)	1 x 50 mL
ENZ CONJ	Enzyme Conjugate (100X Concentrate)	1 vial/150 µL
WASH	Wash Buffer (20X Concentrate)	1 x 50 mL
SUB	Substrate Solution	1 x 12 mL
STOP	Stop Solution	1 x 12 mL

E.2. Materials required but not provided

Micropipettes and disposable tips
Standard laboratory glassware for buffer and reagent preparation
Distilled or deionized water
Polypropylene microtubes
Microplate reader (capable of reading A_{450} and A_{630})

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes. In case of contact with eyes or skin, flush immediately with water and contact a medical professional.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Each calibrator and sample should be assayed in duplicate.
2. Given the sample volumes required (5 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
3. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. The samples should be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles. Samples with excessive hemolysis should not be used. *Note: Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.*

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture.
2. Calibrator
   The calibrator must be reconstituted with 1mL of distilled or deionized water. The calibrator, once reconstituted, is at a concentration of 12.20 µg/mL, and it needs to be diluted with 1X Diluent to prepare working standards as described in **I.2.** Aliquot and store any remaining calibrator at -20°C for future use.
3. Diluent (5X Concentrated)
   The diluent has to be diluted 1:5 with distilled or deionized water prior to use. For example, 50 mL of diluent must be diluted with 200 mL of distilled or deionized water. Diluent is stable for at least one week after dilution stored at 2-8°C. Prepare only as needed.
4. Enzyme Conjugate (100X Concentrated)
   The Enzyme Conjugate has to be diluted 1:100 with 1X Diluent prior to use. For example, 10 µL of the enzyme must be diluted with 990 µL of 1X Diluent. Mix gently and avoid foaming. The Enzyme Conjugate is stable for up to 1 hour when stored in the dark. Accordingly, Enzyme Conjugate should be prepared only as needed just prior to use.
5. Wash Buffer (20X Concentrated)
   The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 950 mL of distilled or deionized water. Wash buffer is stable for at least one week after dilution at room temperature or at 2-8°C.
6. Substrate Solution
   Provided as ready to use.
7. Stop Solution
   Provided as ready to use.

I.2. Preparation of working standards

1. Pipette 580 µL of 1X diluent and 30 µL of the calibrator (12.20 µg/mL) into a polypropylene microtube labeled 600 ng/mL, and mix thoroughly.
2. Pipette 300 µL of 1X diluent into six polypropylene microtubes labeled 300, 150, 75, 37.5, 18.75, and 9.38 ng/mL.
3. Dispense 300 µL of the 600 ng/mL standard into the 300 ng/mL microtube, and mix thoroughly.
4. Dispense 300 µL of the 300 ng/mL standard into the 150 ng/mL microtube, and mix thoroughly.
5. Dispense 300 µL of the 150 ng/mL standard into the 75 ng/mL microtube, and mix thoroughly.
6. Prepare the remaining standards following a similar dilution scheme.
7. Dispense 600 µL of 1X diluent into one polypropylene microtube labeled 0 ng/mL. You now have working standards of 600, 300, 150, 75, 37.5, 18.75, 9.38, and 0 ng/mL.

Please note: Working standards should be prepared immediately prior to use.

I.3. Dilution of samples

Samples need to be diluted with 1X Diluent for use with the assay.

Serum/Plasma Samples: A sample dilution of 1:50,000 using 5 µL of sample is generally suitable. To prepare the 1:50,000 dilution, first mix 5 µL of sample with 995 µL of 1x Diluent. Next mix 4 µL of the 1:200 dilution with 996 µL of 1x Diluent to achieve the 1:50,000 dilution.

Since IgG levels can vary, dilution ratios may need to be adjusted as appropriate.

I.4. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. In each well, add 100 µL of diluted sample or working standard.
2. Incubate plate for 60 mins at room temperature. Keep plate covered and level.
3. Aspirate well contents and wash four times using 300 µL of 1X Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
4. In each well, add 100 µL of diluted Enzyme Conjugate and mix well by repeated pipetting.
5. Incubate plate for 30 mins at room temperature. Keep plate covered in the dark and level.
6. Aspirate well contents and wash four times using 300 µL of 1X Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. Add 100 µL of the Substrate Solution in each well.
8. Incubate plate for 10 mins in dark room at room temperature.
9. Stop the reaction by adding 100 µL of Stop Solution.
10. Measure absorbance as soon as possible, and within 30 mins, using a plate reader (measure A_{450} values and subtract A_{630} values).

I.5. Determining the IgG concentration

1. Using computer software, construct the IgG calibration curve by plotting the blank corrected mean absorbance value for each standard on the Y axis versus the corresponding Mouse IgG concentration on the X axis. Blank corrected values are determined by subtracting the mean absorbance value of the blank from the mean absorbance value for each standard. A four parametric logistic (4-PL) curve fit or second order polynomial (quadratic) are suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. Mouse IgG concentrations in the samples are interpolated using the calibration curve and blank corrected mean absorbance values for each sample. For diluted samples, the values obtained must be multiplied by the dilution factor (ie. 50,000) to obtain the final IgG concentration (expressed in ng/mL).
   Note: *Samples with high mouse IgG concentrations (ie. fall above the range of the assay) should be further diluted and rerun.*

J. Performance characteristics

J.1. Assay range

The Mouse IgG ELISA Kit has an assay range from 9.38 - 600 ng/mL.

J.2. Sensitivity

The assay has an average sensitivity of 2.1 ng/mL

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