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ab273150 Mouse IgG ELISA kit - 30 minutes

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Mouse IgG ELISA kit datasheet:

www.abcam.com/ab273150

(use www.abcam.cn/ab273150 for China, or www.abcam.co.jp/ab273150 for Japan)

For characterizing mouse IgG in cell culture supernatants or ascitic fluids.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

The Mouse IgG quantification Kit (ab273150) provides a rapid and easy method (one step ELISA) for the quantitative determination of mouse IgG in cell culture supernatant and ascitic fluid.

The kit includes ready-to-use reagents necessary to analyze up to 90 samples in 30 min. Buffer solutions are color coded in order to simplify pipetting steps.

A polyclonal antibody specific to mouse IgG (H+L) is pre-coated onto microwells. Samples and standards are pipetted into microwells and mouse IgG present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated anti-mouse IgG (H+L) antibody is pipetted and incubated simultaneously with samples. After washing microwells in order to remove any non specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of mouse IgG in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Sensitivity

The detection range is from 20 ng/ml to 1900 ng/ml.

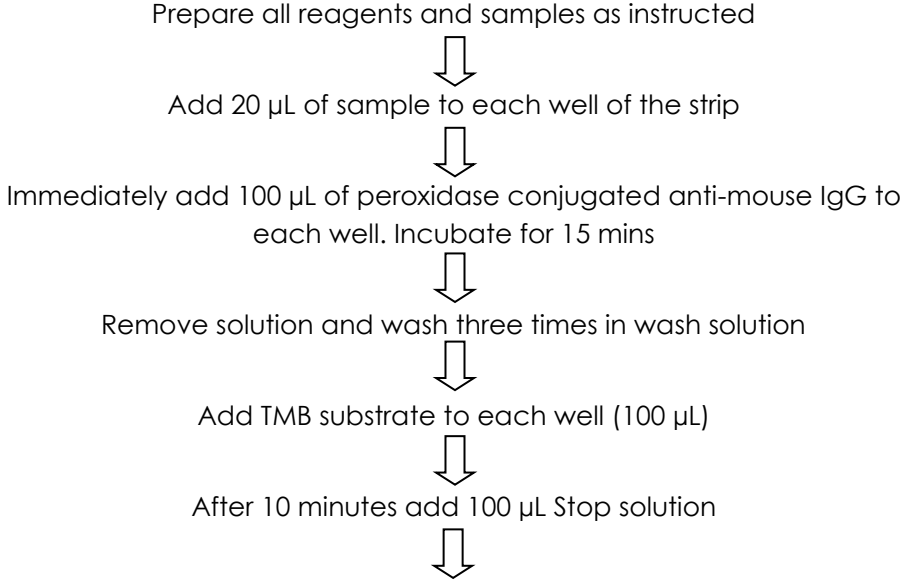
The detection threshold is 6 ng/ml.

Specificity

The method enables the detection of all IgG (IgG3 quantification requires a specific standard curve).

Cross reactions (determined by ELISA) are < 1% for Human IgG, < 1.5% for Cow IgG, < 2% for Goat IgG, < 5 % for Swine IgG and < 15 % for Guinea Pig and Rat IgG. The cross reaction with human serum and fetal calf serum is typically below 0.2%.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt. Kit has a storage time of 12 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	1 x96 tests	10 X 96 tests	Storage Condition
Pre-coated microwells strips	6 strips of 16 microwells	60 strips of 16 microwells	+4°C
Sample Diluent	30 mL	500 mL	+4°C
Detection antibody	12 mL	120 mL	+4°C
TMB Substrate	12 mL	120mL	+4°C
Stop Solution	12 mL	120 mL	+4°C
Mouse IgG Standards	6 X 0.3 mL	6 X 1 mL	+4°C

Note: This ELISA kit will soon contain the "Easy View" colored reagents. The Standard diluent buffer will now be red, and the Streptavidin-HRP Diluent will be green. Please note that while stock lasts you may still receive colorless diluents. This change does not impact the results provided by the kit or the assay procedure.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- ELISA plate washer
- Wash solution (H_20, 0.05% Tween 20)
- Standard microplate reader - capable of reading absorbance at 405 nm and 620nm.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample and reagent.
- Pipette samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

– Equilibrate all reagents to room temperature (25°C or 37°C) prior to use. The kit contains enough reagents for 100 assays.

All reagents are supplied ready to use.

10. Sample Preparation

Dilute the samples in dilution buffer. Recommended dilution factor are indicated in the following table:

Samples	Recommended dilutions
Cell culture supernatant	1/100
Miniperm, CELLine supernatant	1/1000
Ascitic fluid	1/10,000

11. Assay Procedure

– Equilibrate all prepared reagents to desired assay temperature prior to use.

11.1 Transfer 20 µL of diluted samples in each well of the strip

11.2 Immediately add 100 µL of Detection antibody to each well. Mix gently until obtaining a homogeneous purple color.

11.3 Incubate at room temperature for 15 minutes.

11.4 After incubation, remove the solution and wash the wells three times with 300 µL of wash solution.

11.5 Add 100 µL of TMB substrate to each well. Tap plate briefly to mix. Incubate for 10 minutes at room temperature.

11.6 After incubation add 100 µL of stop solution to each well.

11.7 Results can be seen directly or read the absorbance with a microplate reader at 450 nm and 620 nm.

12. Calculations

12.1 Calculate the average absorbance values for each standard.

12.2 Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding standard concentration on the horizontal axis.

12.3 The mouse IgG concentration in the sample can be calculated by interpolation between standards points on the curve.

ΔNote: The mean absorbance of the 0 ng/ml standard should be below 0.1 AU (absorbance unit). Maximal absorbance (1900 ng/ml standard) should be around 1.6 to 2.2 AU, depending of the operating temperature

ΔNote: It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.

- If the absorbance value is equivalent or higher than the 1900 ng/ml standard

13. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

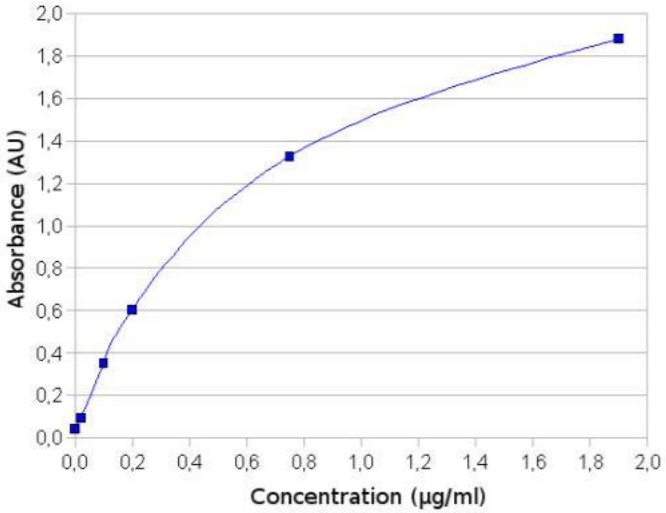


Figure 1. Example of Mouse IgG quantitative kit Standard curve

14. Performance Characteristics

- **Precision**

Intra-Assay				
Sample	Dilution	Mean (ng/ml)	SD	No of measures
A	1/100	10.63	5.33	9
B	1/100	11.56	7.47	9
C	1/100	22.61	8.48	9
D	1/100	28.88	10.03	9
E	1/100	66.82	8.39	9
F	1/100	75.92	9.9	9
G	1/100	102.47	10.38	9

Inter-Assay			
Sample	Dilution	SD (%)	No of measures
Supernatant	1/250	3.45	30
Supernatant	1/500	2.99	30
Supernatant	1/1000	4.97	30

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/en-us/contact-us

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)