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ab151276
Mouse IgG ELISA Kit

For the quantitative measurement of mouse IgG concentrations in serum and cultured media.

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

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1. Overview

Principle: ab151276, an IgG mouse ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of mouse IgG in mouse serum, plasma and supernatant from cell cultures.

This assay employs a mouse IgG specific antibody coated onto plate strips. Standards and samples are pipetted into the wells and IgG present in the sample is bound to the wells by the immobilized antibody. The wells are washed and an HRP-conjugated anti-mouse IgG detector antibody is added. After washing away unbound detector antibody, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IgG bound. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Background: There are five classes of mammalian immunoglobulins: IgA, IgD, IgE, IgM, and IgG. IgG is the most abundant immunoglobulin and is equally distributed in blood and tissue. In mice, the IgG class is further divided into four subclasses: IgG1, IgG2a/ IgG2c (strain specific), IgG2b, and IgG3. The general immunoglobulin structure is composed of four polypeptide chains, two heavy and two light chains linked together and to each other by disulfide bonds, creating a tetrameric quaternary structure. The resulting tetramer creates two identical halves which together form a Y like structure. While the amino-terminal portions that exhibits highly variable amino-acid composition are involved in antigen binding, the C terminal constant parts are involved in complement binding, placental passage and binding to cell membrane. IgG is involved in response to a foreign antigen. The presence of IgG usually signifies a mature antibody response. IgG has a molecular weight of about 150 kDa, it can bind to many pathogens and also plays an important role in antibody-dependent cell-mediated cytotoxicity. Typically, mouse serum and plasma samples contain about 7 to 10 mg/ml of IgG.

The widespread use of hybridoma technology for the production of mouse monoclonal antibodies has created the need for a fast and

simple procedure for quantifying antibody production in vitro (e.g., culture supernatant) or in vivo (e.g., ascites). An accurate determination of immunoglobulin levels in the hybridoma-culture supernatant is essential to study the effect of drugs or physical parameters on hybridoma growth, or the level of immunoglobulin secretion. Due to idiotypic and allotypic variations, this product may not accurately quantitate supernatants from monoclonals. To quantify monoclonal hybridoma supernatants, please using isotype specific IgG mouse ELISA kits i.e. IgG1 mouse ELISA (ab133045), IgG2a mouse ELISA (ab133046), IgG2b mouse ELISA (ab136941) and IgM mouse ELISA (ab133047).

2. Protocol Summary

Equilibrate all reagents to room temperature. Prepare the reagents, samples, and standards as instructed.

Add 50 µL standard or sample to each well used. Incubate 2 hours at room temperature.

Aspirate and wash each well two times. Add 50 µL prepared 1X HRP labeled Detector Antibody to each well. Incubate 1 hour at room temperature.

Aspirate and wash each well three times. Add 50 µL HRP Development Solution to each well. Incubate 10 minutes at room temperature in the dark.

Add 50 µL Stop Solution and read at 450 nm.

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 at least 6 months from receipt, providing components have not been reconstituted.

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.

After reconstitution, the standard should be stored at -80°C.

Unused microplate strips should be returned to the pouch containing the desiccant and resealed.

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	Quantity	Storage Condition
20X Buffer	20 mL	4°C
10X Blocking Buffer	6 mL	4°C
Normal Mouse IgG Standard (Lyophilized)	1 µg	4°C
IgG Mouse Microplate (8 x 12 antibody coated well strips)	96 Wells	4°C
10X GAM (H+L)-HRP Detector Antibody	1 mL	4°C
HRP Development Solution	6 mL	4°C
Stop Solution	12 mL	4°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 nm.
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Optional plate shaker for all incubation steps.

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.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Equilibrate all reagents to room temperature (18-25°C) before use.

9.2 Prepare 1X Wash Buffer by adding 20 mL 20X Buffer to 380 mL nanopure water. Mix gently and thoroughly.

9.3 Prepare 1X Incubation Buffer by adding 6 mL 10X Blocking Buffer to 54 mL 1X Wash Buffer. Unused 1X Incubation Buffer may be stored at -20°C for 6 months after performing the ELISA.

9.4 Prepare the 1X HRP Labelled Detector Antibody by diluting the 10X GAM (H+L)-HRP Detector Antibody 10-fold with 1X Incubation Buffer immediately prior to use. Prepare 500 µL for each 8 well strip used.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

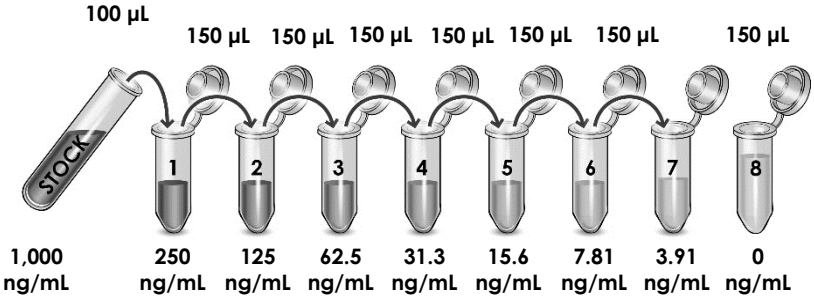
ΔNote: It is strongly recommended to prepare a dilution series of the standard Mouse IgG. The relative levels of mouse IgG in other experimental samples can be interpolated from within this positive control sample series.

10.1 Reconstitute the Normal Mouse IgG standard sample by adding 1,000 µL of 1X Incubation Buffer. Mix thoroughly and gently. Hold at room temperature for 10 minutes and mix gently. This is the 1,000 ng/mL **Stock Standard** Solution.

10.1.1 Label eight tubes, Standards 1– 8.

10.1.2 Add 300 µL 1X Incubation Buffer into tube number 1 and 150 µL of 1X Incubation Buffer into numbers 2-8.

10.1.3 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. Sample Preparation

11.1 Preparation of serum and plasma samples

1X Incubation Buffer is used for dilution of serum/plasma samples. Serum/plasma samples require at least 10,000-fold dilution.

Example:

To prepare10,000-fold diluted samples first add 10 µL of serum/plasma into a tube with 990 µL 1X Incubation Buffer to prepare a 100-fold diluted sample. Mix thoroughly and then pipette 10 µL of the 100-fold diluted sample into a tube with 990 µL 1X Incubation Buffer to prepare a final 10,000-fold diluted sample.

ΔNote: levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

11.2 Preparation of supernatant from hybridoma cell culture

1X Incubation Buffer is used to dilute the supernatant from hybridoma cell culture. The samples should be diluted to within the working range of the assay in 1X Incubation Buffer, as appropriate. As a guide, typical ranges of sample concentration for commonly used sample types are shown below:

Typical working ranges	
Sample Type	**Range**
Mouse IgG	1-1000 ng/mL
Serum/plasma	1:1000 – 1: 1,000,000 fold dilution
Conditioned media from cell culture	1:100- 1: 100,000 fold dilution

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

12.1 Prepare all reagents, working dilutions of controls and samples as directed in the previous sections.

12.2 Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and seal.

12.3 Add 50 µL of each sample per well. It is recommended to include a dilution series of a positive control sample (section 10), as well as tested sample. Also include a no material control (1X Incubation Buffer) as a zero standard.

12.4 Cover/seal the plate and incubate for 2 hours at room temperature. If available use a plate shaker for all incubation steps at 400 rpm.

12.5 Aspirate each well and wash, repeat this once more for a total of two washes. Wash by aspirating or decanting from wells then dispensing 300 µL 1X Wash Buffer into each well as described above. Complete removal of liquid at each step is essential to good performance. After the last wash, remove the remaining buffer by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

12.6 Immediately prior to use prepare sufficient (500 µL/strip used) 1X HRP Labeled Detector Antibody (step 9.4) in 1X Incubation Buffer. Add 50 µL 1X HRP labeled Detector Antibody to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker at 400 rpm.

12.7 Repeat the aspirate/wash procedure above, however, performing a total of three washes.

12.8 Add 50 µL of HRP Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To

avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

12.9 Add 50 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

12.10 Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of HRP Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

12.11 **Note**: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

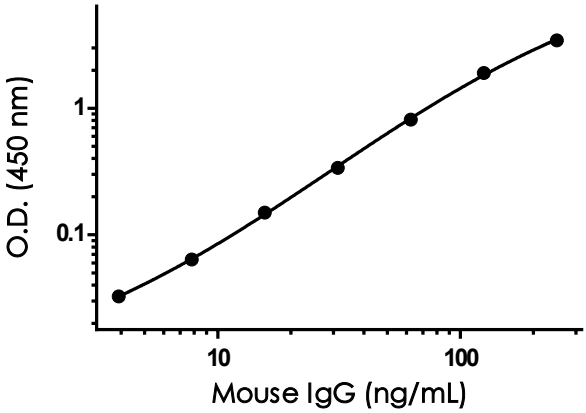
12.12 Analyze the data as described below.

13. Calculations

Average the duplicate positive controls readings and plot against their concentrations after subtracting the zero standard reading. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four-parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4-parameter logistic). Read relative mouse IgG concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

14. Typical Data

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



Standard Curve Measurements			
Concentration (ng/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.058	0.058	0.058
3.91	0.091	0.090	0.091
7.81	0.122	0.122	0.122
15.6	0.200	0.217	0.209
31.3	0.382	0.412	0.397
62.5	0.850	0.897	0.873
125	1.937	1.988	1.963
250	3.467	3.533	3.500

Figure 1. Example of Mouse IgG standard curve in 1X Incubation Buffer. The IgG standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

TYPICAL EXPERIMENTAL RESULTS - for demonstration only.

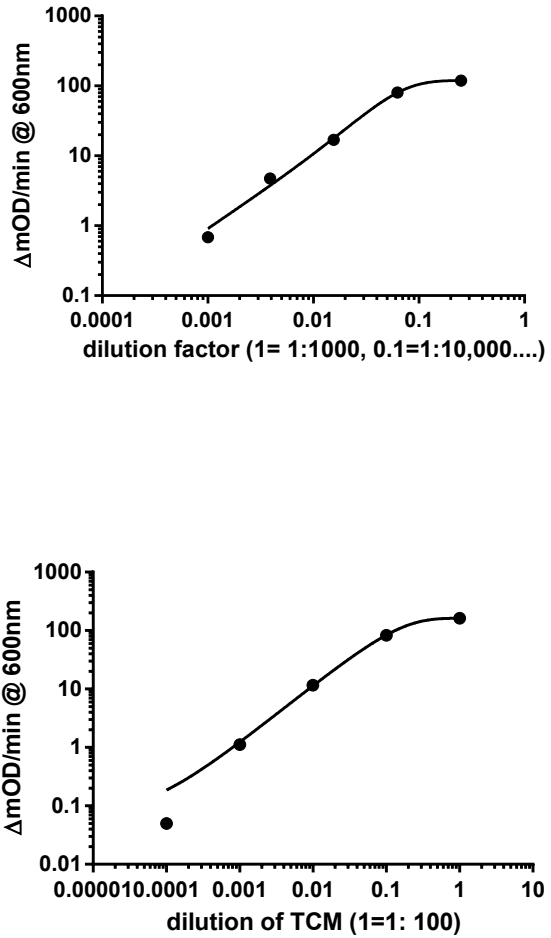


Figure 2: (top) Example dilution series of normal mouse serum (NMS) in the working range of the assay. (bottom) Example dilution series of a mouse hybridoma cell culture media (TCM) in the working range of the assay.

15. Typical Sample Values

SENSITIVITY –

Determined minimum detectable dose of mouse IgG (zero dose n=16 + 2 standard deviations) is 2.2 ng/ml.

PRECISION –

Parameter	CV%
Intra (n=3)	3.3
Inter (n=4)	2.3

RECOVERY –

Normal Mouse IgG	Average Recovery (%)
10% Bovine serum	89
10% Goat serum	96
50% media (0F HGDMEM)	85

Linearity of Dilution

Linearity of dilution determined by comparing dilution series of normal mouse serum (starting concentration is 1:1000-fold dilution) to the concentration of normal mouse IgG (ng/ml).

Mouse serum	% Expected Value
1:1	100
1:4	127
1:16	90
1:64	122
1:256	80

16. Assay Specificity

Species – specifically reactive with mouse IgG, but also weakly reactive with rat serum. Not reactive with bovine serum or goat serum. Other species untested.

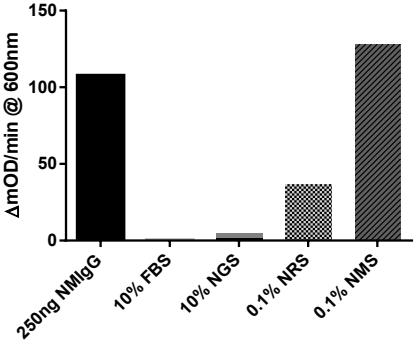


Figure 3: Demonstration of the tested species specificity.

Component requirements were determined by skipping one critical component.

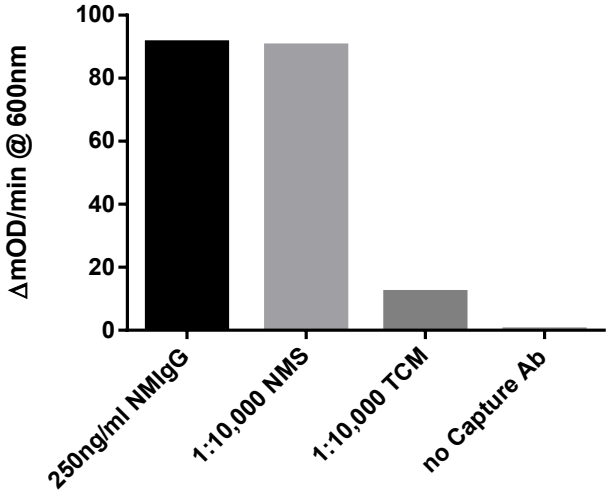


Figure 4: Demonstration of the component requirement test.

17. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)