ab133045 – IgG_1 Mouse ELISA Kit

Instructions for Use

For quantitative detection of Mouse IgG_1 in culture supernatants and serum.

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

Version v7a, Last Updated 26 February 2024

Table of Contents

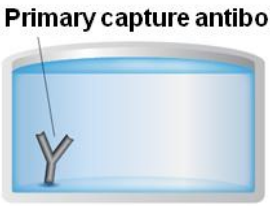
1. BACKGROUND

Abcam's Mouse IgG_1 *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Mouse IgG_1 in Culture supernatants and Serum.

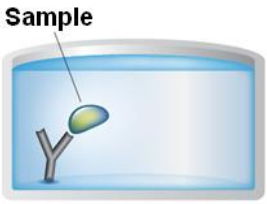
IgG_1 specific antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and along with an HRP-conjugated IgG_1 detection antibody and the microplate is then incubated at room temperature. After the removal of unbound proteins by washing, TMB is used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a colored product that changes after adding acidic stop solution. The density of coloration is directly proportional to the IgG_1 amount of sample captured in plate.

IgG is divided into four subclasses: IgG_1, IgG_2, IgG_3, and IgG_4. IgG_1 is the most abundant immunoglobulin found in the blood. It is a glycoprotein which consists of two identical heavy chains (50 kDa each) and two identical light chains (25 kDa each), to give a combined mass of approximately 150 kDa. The chains are held in place by covalent disulfide bonds. Each light chain contains two immunoglobulin (Ig) domains, while the heavy chains contain four Ig domains each. In the middle of each heavy chain is a relative varying portion called the "hinge region" which is unique to each IgG. This region allows for molecular flexibility and sets IgG_1 apart from its IgG counterparts. IgG_1 properties and functions include neutralization, opsonization, activation of the complement system, diffusion into extravascular sites and crossing the placenta.

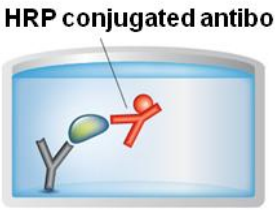
2. ASSAY SUMMARY



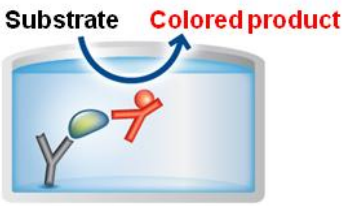
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



Add standard or sample to each well used. Incubate at room temperature.



Aspirate and wash each well. Add prepared HRP labeled secondary detector antibody. Incubate at room temperature



Aspirate and wash each well. Add TMB Substrate Solution to each well. Immediately begin recording the color development

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- Stop Solution 2 is a 1 normal (1N) hydrochloric acid solution. This solution is caustic; care should be taken in use.
- The activity of the Mouse IgG1 Conjugate Concentrate (Horseradish peroxidase conjugate) is affected by nucleophiles such as azide, cyanide and hydroxylamine.
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.

4. STORAGE AND STABILITY

All components of this kit, **except the conjugate concentrate**, are stable at 4°C until the kit's expiration date. The conjugate concentrate must be stored at -20°C upon receipt.

Refer to list of materials supplied for storage conditions of individual components.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition
Goat anti-mouse IgG Microplate (12 x 8 wells)	96 Wells	4°C
Mouse IgG1 Conjugate Diluent	6 mL	4°C
Mouse IgG_1 Conjugate Concentrate (100X concentrate of goat anti-mouse IgG1 conjugated to Horseradish peroxidase)	0.07 mL	-20 °C
Mouse IgG_1 Standard	250 µL	4°C
Assay Buffer 13 Concentrate	50 mL	4°C
20X Wash Buffer Concentrate	100 mL	4°C
TMB Substrate	12 mL	4°C
Stop Solution 2	11 mL	4°C
Plate Sealer	2 Units	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Standard microplate reader - capable of reading at 450 nm, preferably with correction between 570 and 590 nm
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Microplate Shaker
- Absorbent paper for blotting
- Washing buffer, (see Section 9 for recipes)
- Deionized water

7. 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

8. TECHNICAL HINTS

- Standards can be made up in either glass or plastic tubes
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent
- Pipette standards and samples to the bottom of the wells
- Add the reagents to the side of the well to avoid contamination
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results
- It is important that the matrix for the standards and samples be as similar as possible. Mouse IgG_1 samples diluted with Assay Buffer 13 should be run with a standard curve diluted in the same buffer. Serum samples should be evaluated against a standard curve run in Assay Buffer 13 while culture supernatant samples should be read against a standard curve diluted in the same complete but non-conditioned media
- **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**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 Mouse IgG_1 HRP Conjugate

Prepare conjugate by diluting the Conjugate Concentrate 1:100 into Conjugate Diluent. For example, dilute 60 µL of the 100x Conjugate Concentrate with 5.94 mL of Conjugate Diluent. Prepare only what is needed for each day's experiment and discard any remaining diluted conjugate.

1X Assay Buffer

Prepare the Assay Buffer by diluting 50 mL of the supplied Concentrate in 450 mL of deionized water. Mix thoroughly and gently. This can be stored are room temperature until the kit expiration, or for 3 months, whichever is earlier.

9.2 1X Wash Buffer

Prepare the 1X Wash Buffer by diluting 50 mL of the 20X Wash Buffer Concentrate in 950 mL of deionized water. Mix thoroughly and gently.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Diluted standards should be used within 60 minutes of preparation.

10.1 Allow the 5,000 ng/mL IgG1 Stock Standard solution to warm to room temperature. Diluted standards should be used within 60 min of preparation. Discard any unused diluted standards after that.

10.2 For **Mouse serum** samples dilute the IgG_1 standards with Assay Buffer 13.

10.3 For **culture supernatants** sample dilute the IgG1 standards with culture media.

10.4 Label seven tubes with numbers 1 – 7.

10.5 Add 250 µL of appropriate diluent to tubes 2 – 6.

10.6 Prepare a 250 ng/mL **Standard 1** by adding 25 µL of the 5,000 ng/mL Stock Standard to 475 µL of the appropriate diluent to **tube 1**. Mix thoroughly and gently.

10.7 Prepare **Standard 2** by transferring 250 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.8 Prepare **Standard 3** by transferring 250 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.9 Using the table below as a guide, repeat for tubes 4 through 6.

10.10 **Standard 7** contains no protein and is the **blank control**.

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Stock	25	475	5,000	250
2	Standard 1	250	250	250	125
3	Standard 2	250	250	125	62.5
4	Standard 3	250	250	62.5	31.25
5	Standard 4	250	250	31.25	15.62
6	Standard 5	250	250	15.62	7.81
7	-	-	250	-	0

11. SAMPLE COLLECTION AND STORAGE

- The IgG_1 (mouse), ELISA is compatible with mouse IgG_1 culture supernatants and serum
- Samples diluted sufficiently into the proper diluent can be read directly from a standard curve. Culture supernatants and serum are suitable for use in the assay
- Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens
- Samples in the majority of culture media, including fetal bovine serum, can also be read in the assay provided the standards have been diluted into the culture media instead of Assay Buffer 13. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of mouse IgG_1 in the appropriate matrix
- Samples must be stored frozen to avoid loss of bioactive mouse IgG_1. If samples are to be run within 24 hours, they may be stored at 4°C. Otherwise, samples must be stored frozen at -70°C to avoid loss of bioactive mouse IgG_1
- Excessive freeze/thaw cycles should be avoided. Prior to assay, frozen sera should be brought to room temperature slowly and gently mixed by hand
- Do not thaw samples in a 37 °C incubator. Do not vortex or sharply agitate samples

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use**
- **It is recommended to assay all standards, controls and samples in duplicate**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Add 50 µL of Standards 1 through 7 into the appropriate wells.

13.3 Add 50 µL of the Samples into the appropriate wells.

13.4 Add 50 µL of Mouse IgG_1 HRP Conjugate to each well

13.5 Tap the plate gently to mix the contents, and seal with the plate sealer.

13.6 Incubate the plate at room temperature on a plate shaker for 1 hour. The plate may be covered with the plate sealer provided.

13.7 Empty the contents of the wells and wash by adding 300 µL of 1X Wash Buffer 13 to every well. Repeat the wash 3 more times for a total of 4 Washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.8 Add 100 µL of the Substrate solution to every well. Incubate at room temperature for 30 minutes on a plate shaker.

13.9 Add 100 µL Stop Solution 2 into each well. The plate should be read immediately.

13.10 Read the O.D. absorbance at 450 nm, preferably with correction between 570 and 590 nm.

14. CALCULATIONS

A four parameter algorithm (4PL) 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). Interpolate protein 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.

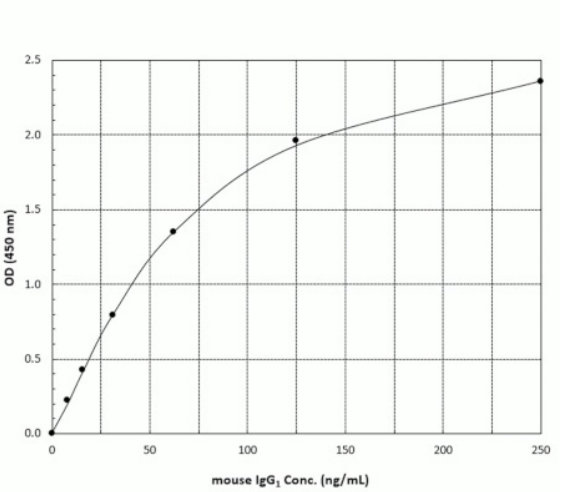
- Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average blank control OD from the average OD bound:

  Average Net OD = Average Bound OD – Average blank control OD

- Plot the average Net OD for each standard versus IgG1 concentration in each standard. Sample concentrations may be calculated off of Net OD values using the desired curve fitting

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Sample	Mean OD (-Blank)	IgG_1 ng/mL
Standard 1	2.354	250
Standard 2	1.964	125
Standard 3	1.349	62.5
Standard 4	0.793	31.25
Standard 5	0.429	15.62
Standard 6	0.222	7.81
Unknown1	2.044	148.7
Unknown 2	1.731	98.3
Unknown 3	1.136	48.5

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run as Bo, and comparing to the average optical density for sixteen (16) wells run with Standard 6. The detection limit was determined as the concentration of IgG_1 measured at two (2) standard deviations from the zero along the standard curve was found to be 0.116 ng/mL

SAMPLE RECOVERY –

Recovery was determined by IgG_1 into tissue culture media, and mouse serum. Mean recoveries are as follows:

Sample Type	Average % Recovery	Recommended Dilution
Mouse Serum	102.8	1:20,000
Tissue Culture Media	105.3	Neat

LINEARITY OF DILUTION –

A sample containing 100 ng/mL IgG_1 was diluted 4 times 1:2 in the kit Assay Buffer 13 and measured in the assay. The data was plotted graphically as actual IgG_1 concentration versus measured IgG_1 concentration.

The line obtained had a slope of 0.920 and a correlation coefficient of 0.999.

PRECISION –

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of mouse IgG_1 and running these samples multiple times (n=19) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of mouse IgG_1 in multiple assays run over 3 days (n=16). The precision numbers listed below represent the percent coefficient of variation for the concentrations of mouse IgG_1 determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	IgG_1 (ng/mL)	Intra-Assay %CV
Low	49.9	1.2
Medium	99.6	2.7
High	160.4	3.6

	IgG_1 (ng/mL)	Inter-Assay %CV
Low	48.5	3.1
Medium	98.3	4.4
High	148.7	8.1

17. ASSAY SPECIFICITY

CROSS REACTIVITY –

The mouse IgG_1 Isotyping ELISA kit is specific for mouse IgG_1. It has a cross-reactivity of 0.9% with rat IgG_1 and 0.21% with mouse IgG_{2b}. It has less than 0.01% cross-reactivity with human IgG_1 and the following mouse proteins: IgG_{2a}, IgG_3, and IgM.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed

19. 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)