ab133046 – IgG_{2a} Mouse ELISA Kit

Instructions for Use

For quantitative detection of Mouse IgG_{2a} in Tissue Culture Media and serum.

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

Version v2d Last Updated 19 February 2024

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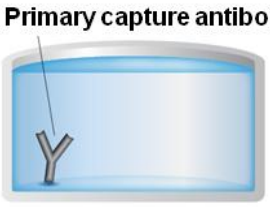
1. BACKGROUND

Abcam's Mouse IgG_{2a} *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Mouse IgG_{2a} in Tissue Culture Media and Serum.

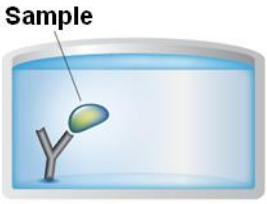
IgG_{2a} specific antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and along with an HRP-conjugated IgG_{2a} detection antibody and the microplate is then incubated at room temperature. After the removal of unbound proteins by washing, TMB is used to 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_{2a} amount of sample captured in plate.

IgG2 is divided into two subclasses; IgG_{2a} and IgG_{2b}. It is a glycoprotein which consists of two identical heavy chains (50kDa 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_{2a} apart from its IgG counterparts. Properties of IgG_{2a} include neutralization of toxins and diffusion in the extracellular space.

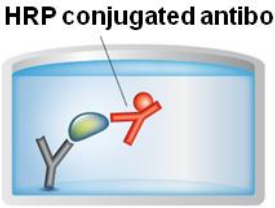
2. ASSAY SUMMARY



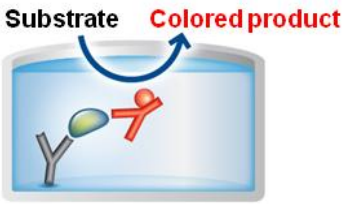
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



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 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

Store the kit at 4°C immediately upon receipt, apart from the IgG_{2a} Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Goat anti-mouse IgG Microplate (12 x 8 wells)	96 Wells	4ºC
Mouse IgG_{2a} HRP Conjugate	5 mL	4ºC
Mouse IgG_{2a} Standard	2 vials	-20ºC
Assay Buffer 13 Concentrate	50 mL	4ºC
20X Wash Buffer Concentrate	100 mL	4ºC
TMB Substrate	10 mL	4ºC
Stop Solution 2	10 mL	4ºC
Plate Sealer	2	4ºC

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
- 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_{2a} 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 Tissue Culture 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 **IgG_{2a} Horseradish Peroxidase Conjugate**

Allow the IgG_{2a} Horseradish Peroxidase Conjugate to equilibrate to room temperature. Any unused conjugate should be stored at +4°C

9.2 **1X Assay Buffer 13**

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 at room temperature until the kit expiration, or for 3 months, whichever is earlier.

9.3 **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**.**

9.4 **Mouse IgG2a Standard**

Allow the Mouse IgG2a Standard to equilibrate to room temperature. Avoid repeated freeze-thaw cycles

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Reconstitution of the IgG_{2a} standard should be prepared no more than 1 hour prior to the experiment. Diluted standards should be used within 60 minutes of preparation.

Mouse IgG2a Standard (2 x 500ng vials)

10.1 Allow the Mouse IgG2a Standard to equilibrate to room temperature. Avoid repeated freeze-thaw cycles.

10.2 **For mouse serum** samples reconstitute the IgG_{2a} standard by adding 1X Assay Buffer 13 into the vial and vortex. **For culture supernatants** samples reconstitute the IgG_{2a} standard by adding 1 mL of the culture media into the vial and vortex. Wait 5 minutes and vortex again prior to use. This is the 500 ng/mL **Standard Stock Solution** (see table below).

10.3 Label six tubes with numbers 1 – 7.

10.4 Add 250 µL of the diluent used to reconstitute the standard into tubes (1 – 7).

10.5 Prepare a 250 ng/mL **Standard 1** by transferring 250 µL from the **Standard Stock Solution** to tube 1. Mix thoroughly and gently.

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

10.7 Using the table below as a guide, repeat for tubes 3 through 6.

10.8 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	See Step 10.4				250
2	Standard 1	250	250	250	125
3	Standard 2	250	250	125	62.5
4	Standard 3	250	250	62.5	31.3
5	Standard 4	250	250	31.3	15.6
6	Standard 5	250	250	15.6	7.81
7	None	-	250	-	0

11. SAMPLE COLLECTION AND STORAGE

- The IgG_{2a} (mouse), EIA kit is compatible with mouse IgG_{2a} samples in Tissue Culture Media and mouse serum. Samples diluted sufficiently into the proper diluent can be read directly from a standard curve
- Culture fluids 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 Tissue Culture Media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the Tissue 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_{2a} in the appropriate matrix
- Samples must be stored frozen to avoid loss of bioactive mouse IgG_{2a}. 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_{2a}. 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
- **High Dose Hook**

  Due to the binding nature of IgGs, a high dose hook effect will ultimately present at higher IgG concentrations. For this assay the high dose hook effect will become noticeable at standard and sample concentrations above 250 ng/mL. Samples with concentrations above 250 ng/mL after recommended dilution may be outside the linear range of the assay. Therefore, samples diluted to the minimal recommended dilution will need to read within the standard curve range for accurate results.

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 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_{2a} HRP Conjugate to each well.

13.5 Incubate the plate at room temperature on a plate shaker for 1 hour at ~500 rpm. The plate may be covered with the plate sealer provided.

13.6 Empty the contents of the wells and wash by adding 400 µL of 1X Wash Buffer 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.

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

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

13.9 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.

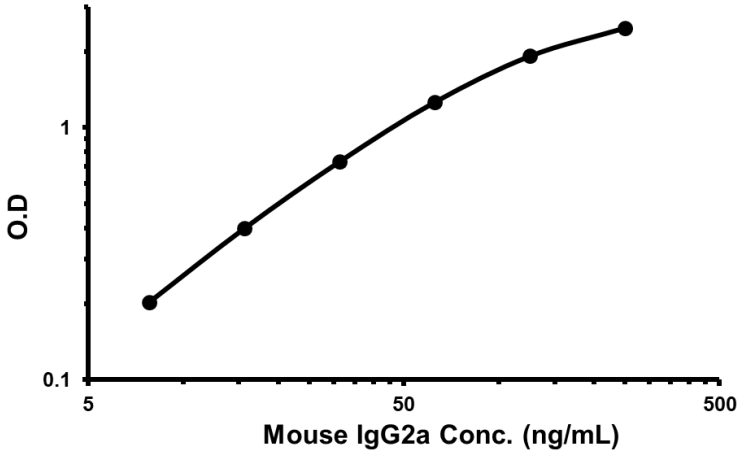
- Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average blank control OD from the average OD bound:

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

- Plot the average Net OD for each standard versus IgG2a 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.



16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The sensitivity, minimum detectable dose of IgG_{2a} using this Abcam ELISA kit was found to be 47.3 pg/mL. This was determined by the average optical density of the 0 pg/mL Standard and comparing to the average optical density for Standard 6. The detection limit was determined as the concentration of IgG_{2a} measured at two standard deviations from the zero along the standard curve.

SAMPLE RECOVERY –

Recovery was determined by IgG_{2a} into tissue culture media, and mouse serum. Mean recoveries are as follows:

Sample Type	Average % Recovery	Recommended Dilution
Tissue Culture Media	98.2	None
Mouse Serum	98.4	≥1:100,000

LINEARITY OF DILUTION –

A sample containing 219 ng/mL IgG_{2a} 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_{2a} concentration versus measured IgG_{2a} concentration.

The line obtained had a slope of 0.9599 and a correlation coefficient of 0.9958.

PRECISION –

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of mouse IgG_{2a} and running these samples multiple times (n=16) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of mouse IgG_{2a} in multiple assays run over 3 days (n=8). The precision numbers listed below represent the percent coefficient of variation for the concentrations of mouse IgG_{2a} determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	IgG_{2a} (ng/mL)	**Intra-Assay %CV**
Low	37.3	1.0
Medium	72	2.8
High	148	5.7

	IgG_{2a} (ng/mL)	**Inter-Assay %CV**
Low	36.7	4.2
Medium	70.3	3.6
High	143	7.5

17. ASSAY SPECIFICITY

CROSS REACTIVITY –

The mouse IgG_{2a} Isotyping EIA kit is specific for mouse IgG_{2a}. There is 0.49% cross-reactivity with mouse IgG_3 and 0.12% cross-reactivity with mouse IgM. There is less than 0.01% cross reactivity with rat IgG_{2a} and the following mouse proteins: IgG_1 and IgG_{2b}.

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

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

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