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.
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1. **BACKGROUND**

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

IgG\textsubscript{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\textsubscript{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\textsubscript{2a} amount of sample captured in plate.

IgG2 is divided into two subclasses; IgG\textsubscript{2a} and IgG\textsubscript{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\textsubscript{2a} apart from its IgG counterparts. Properties of IgG\textsubscript{2a} include neutralization of toxins and diffusion in the extracellular space.
2. ASSAY SUMMARY

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

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

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

**Substrate Colored product**
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**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat anti-mouse IgG Microplate (12 x 8 wells)</td>
<td>96 Wells</td>
<td>4°C</td>
</tr>
<tr>
<td>Mouse IgG$_{2a}$ HRP Conjugate</td>
<td>5 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Mouse IgG$_{2a}$ Standard</td>
<td>2 vials</td>
<td>-20°C</td>
</tr>
<tr>
<td>Assay Buffer 13 Concentrate</td>
<td>50 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>20X Wash Buffer Concentrate</td>
<td>100 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>10 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Stop Solution 2</td>
<td>10 mL</td>
<td>4°C</td>
</tr>
</tbody>
</table>
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 405 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 IgG₂a 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 **IgG₂a Horseradish Peroxidase Conjugate**

Allow the IgG₂a Horseradish Peroxidase Conjugate to equilibrate to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20°C.

9.2 **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 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.
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.

10.1 Allow the 250 ng Mouse IgG$_{2a}$ Stock Standard to equilibrate to room temperature. Avoid repeated freeze-thaw cycles. For mouse serum samples reconstitute the IgG$_{2a}$ standard by adding 1 mL 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 250 ng/mL Standard #1 Solution (see table below).

10.2 Label six tubes with numbers 2 – 7.

10.3 Add 250 µL of the diluent used to reconstitute the standard into every tubes (2 – 7).

10.4 Prepare a 125 ng/mL Standard 2 by transferring 250 µL from tube 1 to tube 2. Mix thoroughly and gently.

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

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

10.7 Standard 7 contains no protein and is the blank control.
## ASSAY PREPARATION

<table>
<thead>
<tr>
<th>Standard</th>
<th>Sample to Dilute</th>
<th>Volume to Dilute (µL)</th>
<th>Volume of Diluent (µL)</th>
<th>Starting Conc. (ng/mL)</th>
<th>Final Conc. (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>See Step 10.1</td>
<td></td>
<td></td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Standard 1</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>125</td>
</tr>
<tr>
<td>3</td>
<td>Standard 2</td>
<td>250</td>
<td>250</td>
<td>125</td>
<td>62.5</td>
</tr>
<tr>
<td>4</td>
<td>Standard 3</td>
<td>250</td>
<td>250</td>
<td>62.5</td>
<td>31.3</td>
</tr>
<tr>
<td>5</td>
<td>Standard 4</td>
<td>250</td>
<td>250</td>
<td>31.3</td>
<td>15.6</td>
</tr>
<tr>
<td>6</td>
<td>Standard 5</td>
<td>250</td>
<td>250</td>
<td>15.6</td>
<td>7.81</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>-</td>
<td>250</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>
11. SAMPLE COLLECTION AND STORAGE

- The IgG<sub>2a</sub> (mouse), EIA kit is compatible with mouse IgG<sub>2a</sub> 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<sub>2a</sub> in the appropriate matrix.

- Samples must be stored frozen to avoid loss of bioactive mouse IgG<sub>2a</sub>. 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<sub>2a</sub>. 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**

  The assay shows no “high dose hook” effect to 625 ng/mL of mouse IgG<sub>2a</sub>. A sample spiked to contain 1,250 ng/mL read as 302 ng/mL. However, elevated levels of mouse IgG<sub>2a</sub> above 625 ng/mL in the sample to be assayed (after any suggested dilution) may read outside the linear range of the assay.
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:

  \[
  \text{Average Net OD} = \text{Average Bound OD} - \text{Average blank control OD}
  \]

- 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.

Sample Mean OD (−blank) IgG2a ng/mL
Standard 1 2.327 250
Standard 2 1.773 125
Standard 3 1.133 62.5
Standard 4 0.652 31.3
Standard 5 0.405 15.6
Standard 6 0.260 7.81
Unknown 1 1.543 99.0
Unknown 2 0.584 26.2
16. **TYPICAL SAMPLE VALUES**

**SENSITIVITY –**

The sensitivity, minimum detectable dose of IgG\(_{2a}\) using this Abcam ELISA kit was found to be 318.8 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:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Average % Recovery</th>
<th>Recommended Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Culture Media</td>
<td>98.2</td>
<td>None</td>
</tr>
<tr>
<td>Mouse Serum</td>
<td>98.4</td>
<td>≥1:100,000</td>
</tr>
</tbody>
</table>

**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 0.952 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\textsubscript{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\textsubscript{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\textsubscript{2a} determined in these assays as calculated by a 4 parameter logistic curve fitting program.

<table>
<thead>
<tr>
<th>IgG\textsubscript{2a} (ng/mL)</th>
<th>Intra-Assay %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>43.4</td>
</tr>
<tr>
<td>Medium</td>
<td>69.8</td>
</tr>
<tr>
<td>High</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>2.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IgG\textsubscript{2a} (ng/mL)</th>
<th>Inter-Assay %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>41.4</td>
</tr>
<tr>
<td>Medium</td>
<td>67.4</td>
</tr>
<tr>
<td>High</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>4.1</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Inaccurate pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td></td>
<td>Improper standards dilution</td>
<td>Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing</td>
</tr>
<tr>
<td>Low Signal</td>
<td>Incubation times too brief</td>
<td>Ensure sufficient incubation times; change to overnight standard/sample incubation</td>
</tr>
<tr>
<td></td>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
</tr>
<tr>
<td>Samples give higher value than the highest standard</td>
<td>Starting sample concentration is too high.</td>
<td>Dilute the specimens and repeat the assay</td>
</tr>
<tr>
<td>Large CV</td>
<td>Plate is insufficiently washed</td>
<td>Review manual for proper wash technique. If using a plate washer, check all ports for obstructions</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Prepare fresh wash buffer</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the kit</td>
<td>Store the all components as directed</td>
</tr>
</tbody>
</table>
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940
www.abcam.com | www.abcam.cn | www.abcam.co.jp

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