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ab133047
IgM Mouse ELISA Kit

For quantitative detection of Mouse IgM in Tissue culture media, serum and ascites fluids.

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

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1. Overview

Abcam's Mouse IgM *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Mouse IgM in Tissue culture media, serum and ascites fluids.

IgM specific antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and along with an HRP-conjugated IgM 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 IgM amount of sample captured in plate.

IgM is one of the most primitive and least specialized immunoglobulins. It is one of the five classes of antibodies found in mammals and it contains μ class heavy chains. The half-life of IgM is 5 days and it has a high molecular weight of 900,000 Daltons, which prevents its passage into extravascular areas. IgM levels are dependent on the extent of antigenic stimulation from the environment. A developing B cell always makes IgM before any other class of antibody. IgM formed in B cells is a four-chain molecule composed of two light chains and two heavy chains. Once IgM molecules are formed in B cells, they are inserted into the plasma membrane where they act as antigen receptors. IgM is also the major antibody class involved in a primary antibody response, during which IgM molecules are secreted into the blood in the form of a pentamer. In a pentamer, five four-chain IgM units are held together by disulfide bonds and a small glycopeptide known as a J (joining) chain, giving the molecule a total of 10 antigen binding sites.

2. Protocol 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.

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.

- 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, apart from the IgM Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles. Kit has a storage time of 1 year 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.

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
Goat anti-mouse IgM Microplate (12 x 8 wells)	96 wells	+4°C
Mouse IgM Horseradish Peroxidase Conjugate	6 mL	+4°C
Mouse IgM Standard	2 vials	-20°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 x 1 unit	+4°C

7. Materials Required, Not Supplied

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

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.
- 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 IgM 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.
- 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.
- The mouse IgM Standard provided should be handled with care.

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 IgM Horseradish Peroxidase Conjugate

Allow the IgM 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. This can be stored at room temperature until the kit's expiration or 3 months, whichever is earlier. Mix thoroughly and gently.

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. This can be stored at room temperature until the kit's expiration date, or for 3 months, whichever comes first. Mix thoroughly and gently.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Reconstitution of the IgM standard should be prepared no more than 1 hour prior to the experiment.
- Diluted standards should be used within 60 minutes of preparation.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Allow the 250 ng Mouse IgM Stock Standard to equilibrate to room temperature. Avoid repeated freeze-thaw cycles. Reconstitute one vial of IgM standard by adding 1 mL of Assay Buffer 13 for mouse serum and ascites fluid samples or add 1 mL of the culture media for culture supernatants samples. Mix thoroughly and gently. This is the 250 ng/mL **Standard #1** Solution (see table below).

10.2 Label 7 tubes with numbers 2 –8.

10.3 Add 500 µL of the diluent used to reconstitute the standard to every tube (2 – 8).

10.4 Prepare a 125 ng/mL **Standard 2** by transferring 500 µL from tube 1 to tube 2. Mix thoroughly and gently.

10.5 Prepare **Standard 3** by transferring 500 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 7.

10.7 **Standard 8** contains no protein and is the Blank control.

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	See Step 10.1			250
2	500 µL Standard #1	500	250	125
3	500 µL Standard #2	500	125	62.5
4	500 µL Standard #3	500	62.5	31.25
5	500 µL Standard #4	500	31.25	15.62
6	500 µL Standard #5	500	15.62	7.81
7	500 µL Standard #6	500	7.81	3.91
8	-	500	-	0

11. Sample Preparation

- The IgM (mouse), EIA kit is compatible with mouse IgM samples in Tissue Culture Media, mouse serum and mouse ascites fluids. Samples diluted sufficiently into the proper diluent can be read directly from a standard curve.
- Culture fluids, serum and ascites fluids 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 IgM in the appropriate matrix.
- Samples must be stored frozen to avoid loss of bioactive mouse IgM. If samples are to be run within 24 hours, they may be stored at 4°C. Otherwise, samples must be stored frozen at -80°C to avoid loss of bioactive mouse IgM. 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 500 ng/mL of mouse IgM. A sample spiked to contain 500 ng/mL read as 462 ng/mL. However, elevated levels of mouse IgM above 500 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.
- We recommend that you 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 and samples into the appropriate wells.

13.3 Add 50 µL of mouse IgM Conjugate to each well

13.4 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.

13.5 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.

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

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

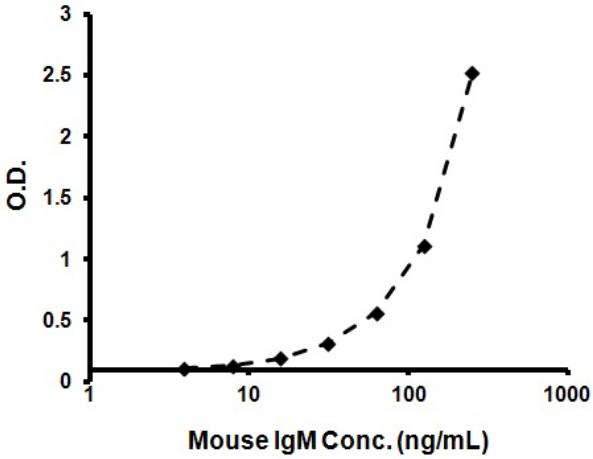
13.8 Read the O.D. absorbance at 450 nm, preferably with correction between 570 and 590 nm.

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.

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)	IgM ng/mL
Standard 1	2.521	250
Standard 2	1.110	125
Standard 3	0.565	62.5
Standard 4	0.314	31.25
Standard 5	0.192	15.6
Standard 6	0.132	7.81
Standard 7	0.110	3.91
Unknown1	1.851	192.1
Unknown 2	0.158	11.7

Figure 1. Example of IgM standard curve.

16. Typical Sample Values

SENSITIVITY –

The sensitivity, minimum detectable dose of IgM using this Abcam ELISA kit was found to be 0.60 ng/mL. This was determined by the average optical density of the 0 ng/mL Standard and comparing to the average optical density for Standard 7. The detection limit was determined as the concentration of IgM measured at two standard deviations from the zero along the standard curve.

SAMPLE RECOVERY –

Recovery was determined by IgM into Tissue culture media, Ascites Fluid and Mouse serum. Mean recoveries are as follows:

Sample Type	Average % Recovery	Recommended Dilution
Mouse Serum	102.8	≥1:20,000
Tissue Culture Media	92.9	None
Mouse Ascites Fluid	94.6	≥1:2,500

LINEARITY OF DILUTION –

A sample containing 204 ng/mL IgM was diluted 4 times 1:2 in the kit Assay Buffer 13 and measured in the assay. The data was plotted graphically as actual IgM concentration versus measured IgM concentration.

The line obtained had a slope of 1.002 and a correlation coefficient of 0.999.

PRECISION –

	IgM (ng/mL)	Intra-Assay %CV
Low	13.9	6.7
Medium	59.8	3.1
High	218.9	2.0

	IgM (ng/mL)	Inter-Assay %CV
Low	11.3	8.5
Medium	58.2	5.0
High	201	5.6

17. Assay Specificity

CROSS REACTIVITY –

The cross reactivities for a number of related compounds was determined by dissolving the cross reactant in Assay Buffer 13 at a concentration ten times greater than the highest standard. These samples were then measured in the mouse IgM assay.

Compound	Cross Reactivity
Mouse IgM	100%
Rat IgM	60%
Human IgM	<0.4%
Mouse IgG1	<0.2%
Mouse IgG2a	<0.1%
Mouse IgG2b	<0.1%
Mouse IgG3	<0.1%

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	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)