ab211646
IgM Monkey ELISA kit

Instructions for use:

for the quantitative measurement of IgM in biological fluid of Monkey.

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

Version v2a Last Updated 25 May 2023

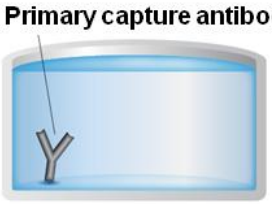
Table of Contents

1. BACKGROUND

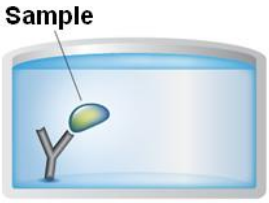
Abcam's IgM Monkey ELISA Kit is an *in vitro* enzyme linked immunosorbent assay (ELISA) (ab211646) for the quantitative measurement of IgM in biological fluid of Monkey.

In this assay the IgM present in samples reacts with the anti-IgM antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-IgM antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound IgM. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromo-genic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of IgM in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of IgM in the test sample. The quantity of IgM in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

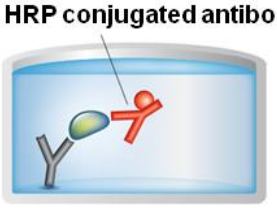
2. ASSAY SUMMARY



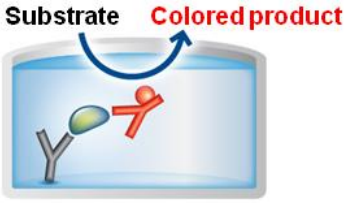
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



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 Chromogen 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 handle 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 4ºC in the dark immediately upon receipt. 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.

Aliquot components in working volumes before storing at the recommended temperature.

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	Amount	Storage Condition (Before Preparation)
IgM Monkey Calibrator (Lyophilized)	1 Vial	- 20°C
IgM Monkey 100X HRP Conjugate	150 µL	4°C
IgM Monkey 5X Diluent	50 mL	4°C
IgM Monkey Antibody coated microwells (12 x 8 well strips)	96 Tests	4°C
20X Wash Buffer Concentrate	50 mL	4°C
TMB Substrate Solution	12 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:

- Precision pipette (2 µL to 200 µL) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H_2O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer

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.
- 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.

- Ensure all reagents and solutions are at the appropriate temperature before starting the assay
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- 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.
- Completely aspirate all solutions and buffers during wash steps.
- When preparing your standards, it is critical to briefly spin down the vial first. The powder may adhere to the cape and not be included in the standard solution resulting in an incorrect concentration. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is an effective technique for thorough mixing of the standard without using excessive mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.

9. REAGENT PREPARATION

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

9.1. IgM Monkey Diluent

The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH_2O).

Store at 2-8°C.

9.2. Wash Solution

The wash solution is supplied as 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals. The 1X Wash Buffer is stable for at least one week from the date of preparation and can be stored at room temperature (16-25°C) or at 2-8°C.

9.3. IgM Monkey HRP Conjugate

Calculate the required amount of 1X IgM Monkey HRP Conjugate solution for each microtitre plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix gentley but thoroughly. Avoid foaming. The working conjugate solution is stable for up to 1 hour when stored in the dark.

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

10.1. Add 1.0 mL of distilled or de-ionized water to the IgM Monkey Calibrator and mix gently until dissolved. The calibrator is now at the concentration stated on the vial.

The reconstituted calibrator should be aliquoted and stored at -20°C if future use is required. Avoid multiple freeze-thaw cycles.

10.2. Label tubes # 1-7

10.3. Prepare **Standard #1** by adding the appropriate volume of 1X Diluent Solution (derived below) to tube # 1. Add 50 µL of

stock IgM Monkey Calibrator to obtain a concentration at 400 ng/mL and mix thoroughly and gently.

*Example:

NOTE: This example is for demonstration purposes only. Please remember to check your calibrator vial for the actual concentration of calibrator provided.

C_S = Starting concentration of reconstituted IgM Monkey Calibrator (variable e.g. 7.0 µg/mL)

C_F = Final concentration of **Standard #1** (400 ng/mL)

V_A = Total volume of IgM Monkey Calibrator to dilute (e.g. 50 µL)

V_D = Total volume of 1X Diluent Solution required to dilute IgM Monkey Calibrator to prepare **Standard #1**

V_T = Total volume of **Standard #1**

D_F = Dilution factor

Calculate the dilution factor (D_F) between the Calibrator and **Standard #1** final concentration:

$$Cs/C_F = D_F$$

$$7{,}000 / 400 = 17.5$$

Calculate the final volume V_D required to prepare **Standard #1** at 400 ng/mL

$$V_A * D_F = V_T$$

$$50 * 17.5 = 875\ \mu L$$

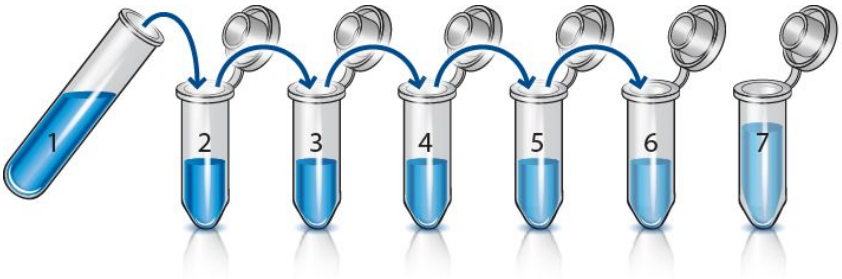
$$V_D = V_T - V_A$$

$$V_D = 875 – 50 = 825\ \mu L$$

To prepare **Standard #1** in tube #1, add 50 µL of IgM Monkey Calibrator to 825 µL of 1X Diluent Solution to obtain a concentration of 400 ng/mL. Mix thoroughly and gently.

Standard #	Volume of Standard (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	See step 10.3				400
2	300 Std. #1	300	600	400	200
3	300 Std. #2	300	600	200	100
4	300 Std. #3	300	600	100	50
5	300 Std. #4	300	600	50	25
6	300 Std. #5	300	600	25	12.5
7 (Blank)	N/A	600	600	0	0

Each dilution has enough amount of standard to set up duplicate readings



11. SAMPLE COLLECTION AND STORAGE

Serum – Blood should be collected by venipuncture and the serum separated from the cells, after clot formation, by centrifugation. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Specimens may be shipped at room temperature and then stored refrigerated at 2-8°C if testing is to take place within one week after collection. If testing is to take place later than one week, specimens should be stored at -20°C. Avoid repeated freeze-thaw cycles.

- **Precautions**

  For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

- **Additives and Preservatives**

  No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

12. SAMPLE PREPARATION

The assay for quantification of IgM in serum requires that each test sample be diluted appropriately before use. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required.

- ***For Example:*** To prepare a 1/30 dilution of your sample, transfer 10 µL of sample to 290 µL of 1X diluent. This gives you a 1/30 dilution. Mix thoroughly.

13. PLATE PREPARATION

General Sample information:

- The 96 well plate strips included with this kit is supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well plate 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

14. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to correct temperature prior to use.**
- **We recommended to assay all standards, controls and samples in duplicate.**
- **Prepare all reagents, working standards, and samples as directed in the previous sections.**

14.1. Pipette 100 µL of each standard, including zero control, in duplicate, into pre designated wells.

14.2. Pipette 100 µL of sample (in duplicate) into pre designated wells.

14.3. Incubate the microtiter plate at room temperature for 60 minutes. Keep plate covered and level during incubation.

14.4. Following incubation, aspirate the contents of the wells.

14.5. Completely fill each well with appropriately diluted 1X Wash Buffer and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by gently striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

14.6. Pipette 100 µL of appropriately prepared 1X IgM Monkey HRP Conjugate to each well. Incubate at room temperature for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

14.7. Wash and blot the wells as described in 14.4 - 14.5.

14.8. Pipette 100 µL of TMB Substrate into each well.

14.9. Incubate in the dark at room temperature for precisely ten (10) minutes.

14.10. After ten minutes, add 100 µL of Stop Solution to each well.

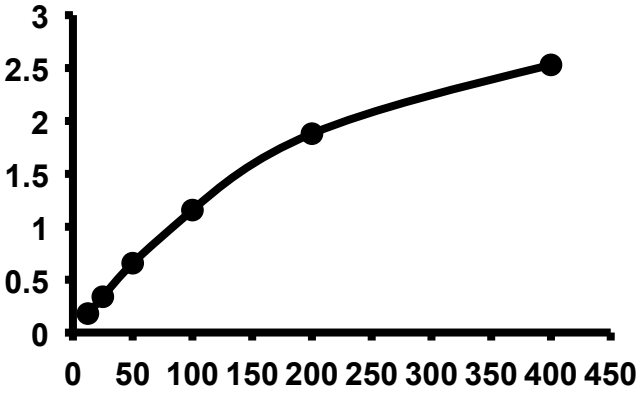
14.11. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacturer's specifications. well.

15. CALCULATIONS

Average the duplicate standard reading for each standard, sample and control blank. Subtract the control blank from all mean readings. Plot the mean standard readings against their concentrations and 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). Extrapolate protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in IgM Monkey Diluent and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

16. TYPICAL DATA

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



Concentration (ng/mL)	Background Adjusted OD Value
12.5	0.180
25	0.339
50	0.656
100	1.158
200	1.88
400	2.53

17. TYPICAL SAMPLE VALUES

SENSITIVITY

Calculated minimum detectable dose = 4.265 ng/mL

RECOVERY

Control Serum Recovery = > 85%

PRECISION

	Intra-Assay	Inter-Assay
%CV	<10	<10

18. INTERFERENCES

These chemicals or biologicals will cause interferences in this assay causing compromised results or complete failure.

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

19. QUICK ASSAY PROCEDURE

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare standard, and prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Pipette 100 µL of each standard, including zero control, in duplicate, into pre designated wells.
- Pipette 100 µL of sample (in duplicate) into pre designated wells.
- Incubate the microtiter plate at room temperature for sixty (20 ± 2) minutes. Keep plate covered and level during incubation.
- Following incubation, aspirate the contents of the wells.
- Completely fill each well with appropriately diluted 1X Wash Buffer and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by gently striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
- Pipette 100 µL of appropriately prepared 1X IgM Monkey HRP Conjugate to each well. Incubate at room temperature for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

- Wash and blot the wells as described in 14.4 - 14.5.
- Pipette 100 µL of TMB Substrate into each well.
- Incubate in the dark at room temperature for precisely ten (10) minutes.
- After ten minutes, add 100 µL of Stop Solution to each well.
- Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacturer's specifications. well.

20. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use provided protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes

	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

21. 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)

All information / detail is correct at time of going to print.