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ab190539 – IgM Rabbit ELISA Kit

For the quantitative measurement of IgM in Rabbit serum and plasma samples.

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

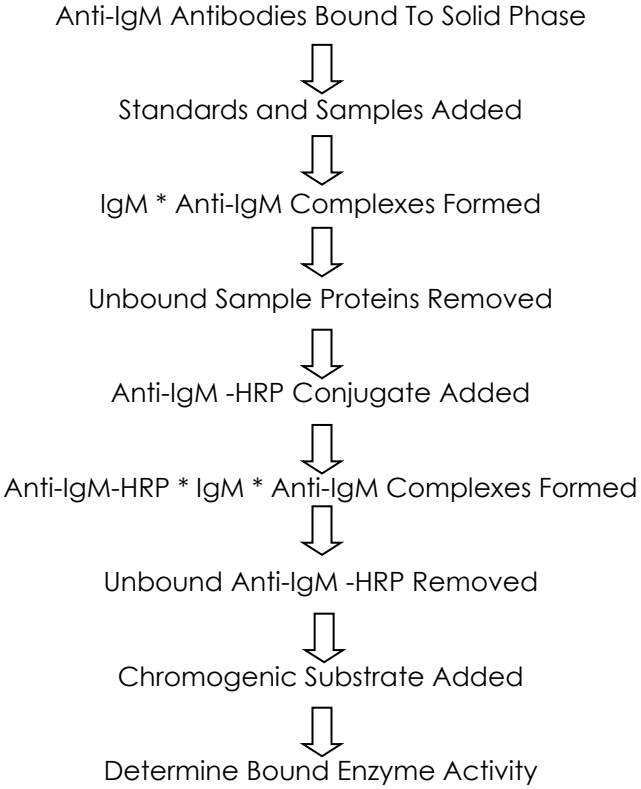
Table of Contents

1. Overview

Abcam's IgM Rabbit ELISA Kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for the measurement of IgM in Rabbit biological samples. If the ELISA is to be used outside the intended use, the user may need to optimize for said use.

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. Protocol Summary



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. Modifications to the kit components or procedures may result in loss of performance.

If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store the components kit at +4-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

5. Limitations

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice

- Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature

- Do not mix or substitute reagents with those from other lots or sources

6. Materials Supplied

Item	Quantity	Storage Condition
IgM Rabbit Calibrator (lyophilized)	1 vial	+4°C
IgM Rabbit HRP Conjugate	1 vial	+4°C (Store in dark)
5X Diluent Concentrate	50 mL	+4°C
IgM Rabbit Antibody pre-coated 96 well microplate (12 x 8 well strips)	96 wells	+4°C
20X Wash Buffer	50 mL	+4°C
Chromogen Substrate Solution	12 mL	+4°C (Store in dark)
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 utilize this assay:

- Precision pipette (2 µL to 200 µL) for making and dispensing dilutions.
- Test tubes.
- Microtitre washer/aspirator.
- Distilled or Deionized H2O.
- Microtitre Plate reader.
- Assorted glassware for the preparation of reagents and buffer solutions.
- Centrifuge for sample collection
- Anticoagulant for plasma collection
- Timer.

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers
- 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
- Complete removal of all solutions and buffers during wash steps

9. Reagent Preparation

– Equilibrate all reagents and samples to room temperature (16-25°C) prior to use.

9.1 Diluent Concentrate

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).

The 1X Diluent Concentrate is stable at 2-8°C for at least one week from the date of preparation.

9.2 Wash Buffer

The wash buffer 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 at 2-8°C for at least one week from the date of preparation.

9.3 IgM Rabbit HRP Conjugate

9.4 Calculate the required amount of 1X IgM Rabbit 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 gently but thoroughly. Dilute immediately before use and protect from light. Avoid foaming.

9.5 IgM Rabbit Antibody microwells

Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal. Store at 2-8°C.

9.6 IgM Rabbit Calibrator (lyophilized)

The calibrator is provided at the concentration stated on the vial. Reconstitute the Rabbit IgM Calibrator with distilled or de-ionized water as specified on the vial and mix gently until dissolved. The amount of calibrator is shown on the vial, and after reconstitution will have a concentration of X µg/mL, where X is the amount on

the vial (the reconstituted calibrator should be aliquoted and stored frozen if future use is intended)..

9.7 Chromogen Substrate Solution

Ready to use as supplied

9.8 Stop Solution

Ready to use as supplied

10. Sample Collection and Storage

1. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing.

2. If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution.

3. The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

4. Known interfering substances - Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

10.1 **Serum** – Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

10.2 **Plasma** – For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

10.3 **Urine** – Collect mid-stream using sterile or clean urine collector. Centrifuge to remove cell debris. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

11. Sample Preparation

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

11.1 **Serum** – Recommended starting dilution is 1/2,000. To prepare a 1/2,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 20 µL into 380 µL of 1X diluent. This gives you a 1/2,000 dilution. Mix thoroughly each stage.

11.2 **Plasma** – Recommended starting dilution is 1/2,000. To prepare a 1/2,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 20 µL into 380 µL of 1X diluent. This gives you a 1/2,000 dilution. Mix thoroughly each stage.

12. Assay Procedure

12.1 All samples and standards should be assayed in duplicates.

12.2 The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

Pipette 100 µL of

Standard 0	(0.0 ng/mL) in duplicate
Standard 1	(6.25 ng/mL) in duplicate
Standard 2	(12.50 ng/mL) in duplicate
Standard 3	(25 ng/mL) in duplicate
Standard 4	(50 ng/mL) in duplicate
Standard 5	(100 ng/mL) in duplicate
Standard 6	(200 ng/mL) in duplicate

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

12.4 Incubate the micro titer plate at room temperature for thirty (30 ± 2) minutes. Keep plate covered and level during incubation.

12.5 Following incubation, aspirate the contents of the wells.

12.6 Completely fill each well with appropriately diluted Wash Solution 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 sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

12.7 Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for ten (10 ± 2) minutes. Keep plate covered in the dark and level during incubation.

12.8 Wash and blot the wells as described in Steps 5/6.

12.9 Pipette 100 µL of TMB Substrate Solution into each well.

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

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

12.12 Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

13. Calculations

13.1 Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.

13.2 Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.

13.3 Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the IgM concentration in original samples.

14. Notes

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)