ELISA PRODUCT INFORMATION & MANUAL

IgM

NBP2-61294

Enzyme-linked Immunosorbent Assay for quantitative detection of Mouse IgM.
For research use only. Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

IgM (mouse), ELISA kit

96 Well Kit

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Description

The IgM (mouse), ELISA kit is a complete kit for the quantitative determintation of mouse IgM in Tissue Culture Media, serum and ascites fluids. Please read the complete kit insert before performing this assay. The kit uses a polyclonal antibody to mouse IgM immobilized on a microtiter plate to bind the mouse IgM in the standards or sample. A mouse IgM Standard is provided in the kit. After a simultaneous incubation with a polyclonal antibody to mouse IgM conjugated to Horseradish peroxidase, which binds to the mouse IgM captured on the plate, the excess reagents are washed out and substrate is added. After a short incubation, the enzyme reaction is stopped and the color generated is read at 450 nm. The measured optical density is directly proportional to the concentration of mouse IgM in either standards or samples. For further explanation of the principles and practices of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

Introduction

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[3]. 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[4].

Precautions

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1. Stop Solution 2 is a 1 normal (1N) hydrochloric acid solution. This solution is caustic; care should be taken in use.
2. The activity of the Horseradish peroxidase conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine.
3. 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. The mouse IgM Standard provided, Catalog No. 80-1124, should be handled with care.

Materials Supplied

1. **Goat anti-mouse IgM Microtiter Plate, One Plate of 96 Wells.**
   A plate using break-apart strips coated with polyclonal antibody specific to mouse IgM.
2. **Assay Buffer 13 Concentrate, 50 mL.**
   Tris buffered saline containing proteins and detergents.
3. **mouse IgM Conjugate, 6 mL.**
   A blue solution of goat anti-mouse IgM conjugated to Horseradish peroxidase.
4. **Wash Buffer Concentrate, 100 mL.**
   Tris buffered saline containing detergents.
5. **mouse IgM Standard, 2 vials.**
   Two vials containing 250 ng each of lyophilized mouse IgM.
6. **TMB Substrate, 10 mL.**
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. Ready to use. **Protect from prolonged exposure to light.**
7. **Stop Solution 2, 10 mL.**
   A 1N solution of hydrochloric acid in water. Keep tightly capped. Caution: **Caustic.**
8. **mouse IgM Isotyping Assay Layout Sheet, 1 each.**
9. **Plate Sealer, 2 each.**

Storage

All components of this kit, **except the Standard**, are stable at 4°C until the kit's expiration date. The Standard **must** be stored at -20°C.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 50 µL and 1,000 µL.
3. Disposable test tubes for dilution of samples and standards.
4. Repeater pipets for dispensing 50 µL.
5. Disposable beakers for diluting buffer concentrates.
6. Graduated cylinders.
7. Plate shaker.
8. Adsorbent paper for blotting.
9. Microplate reader capable of reading at 450 nm, preferably with correction between 570 nm and 590 nm.
10. Graph paper for plotting the standard curve.

Sample Handling

The IgM (mouse), ELISA 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. Please refer to the Sample Recovery recommendations on page 11 for details of suggested dilutions.

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 most 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 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 (after any suggested dilution) may read outside the linear range of the assay.

Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or plastic tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. 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.
8. **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.**
9. **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 and ascites fluids 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. See Reagent Preperation, step #2.**

Reagent Preparation

1. **Wash Buffer**
   Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. **Assay Bufer 13**
   Prepare the Assay Buffer 13 by diluting 50 mL of the supplied concentrate with 450 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

3. **mouse IgM Standards**
   Allow the 250 ng IgM standard to warm to room temperature. Label six 12x75 mm glass tubes #2 through #7. Add 1.0 mL of standard diluent (Assay Buffer 13 or Tissue Culture Media) to the lyophilized mouse IgM vial and vortex. Wait 5 minutes and vortex again prior to use. Pipet 500 µL of standard diluent into tubes #2 through #7. Add 500 µL of reconstituted standard to tube #2 and vortex thoroughly. Add 500 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #7.

   The concentration of mouse IgM in tubes #1 through #7 will be 250, 125, 62.5, 31.25, 15.62, 7.81, and 3.91 ng/mL respectively. See mouse IgM Assay Layout Sheet for dilution details.

   Diluted standards should be used within 60 minutes of preparation.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

Plates will require shaking on an orbital rotor at 500 rpm.

All standards, controls and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4°C.
2. Pipet 50 µL of standard diluent (Assay Buffer 13 or Tissue Culture Media) into the S0 (0 pg/mL standard) wells.
3. Pipet 50 µL of Standards #1 through #7 into the appropriate wells.
4. Pipet 50 µL of the samples into the appropriate wells.
5. Add 50 µL of blue Conjugate to each well, except the Blank.
6. Seal the plate and incubate at room temperature on a plate shaker for 1 hour.
7. Empty the contents of the wells and wash by adding 400 µL of wash solution 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.
8. Pipet 100 µL of Substrate Solution into each well.
9. Incubate for 30 minutes at room temperature on a plate shaker.
10. Pipet 100 µL Stop Solution 2 to each well. This stops the reaction and the plates should be read immediately.
11. Blank the plate reader against the Blank wells, read the optical density at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

Calculation of Results

Several options are available for the calculation of the concentration of mouse IgM in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of mouse IgM can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average Blank OD

2. Plot the Average Net OD for each standard versus mouse IgM concentration in each standard. Approximate a straight line through the points. The concentration of mouse IgM in the unknowns can be determined by interpolation.

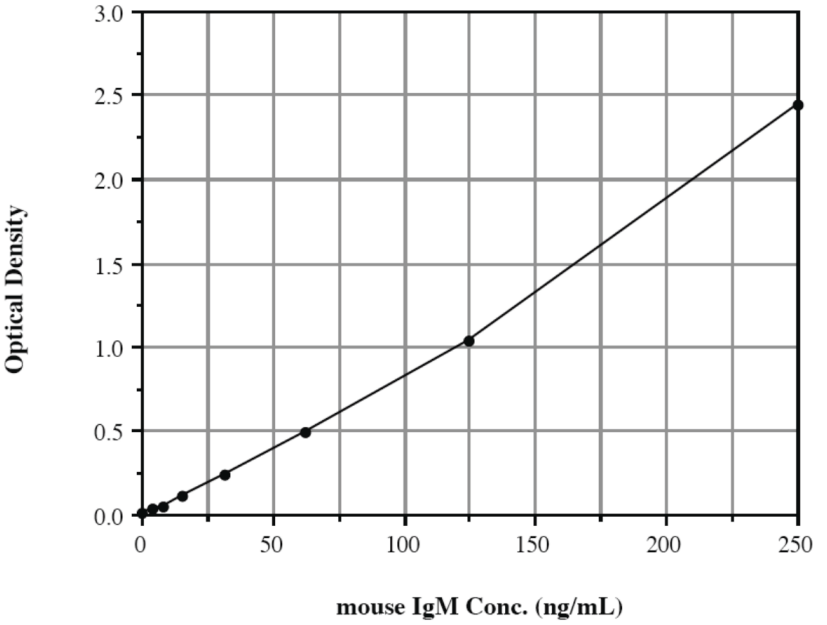
Typical Results

The results shown below are for illustration only and **should not** be used to calculate results from another assay.

Sample	Average OD	Net OD	**m IgM (ng/mL)**
Blank	0.075		
S0	0.085	0.010	**0**
S1	2.521	2.446	**250**
S2	1.110	1.035	**125**
S3	0.565	0.490	**62.5**
S4	0.314	0.239	**31.25**
S5	0.192	0.117	**15.6**
S6	0.132	0.057	**7.81**
S7	0.110	0.035	**3.91**
Unknown 1	1.851	1.776	**192.1**
Unknown 2	0.158	0.083	**11.7**

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate mouse IgM concentrations; each user must run a standard curve for each assay.



Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[5].

Sensitivity

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run with 0 ng/mL Standard, and comparing to the average optical density for sixteen (16) wells run with Standard #7. The detection limit was determined as the concentration of mouse IgM measured at two (2) standard deviations from the 0 ng/mL Standard along the standard curve.

Mean OD for S0 =	0.008 ± 0.002 (20.2%)
Mean OD for Standard #7 =	0.034 ± 0.004 (10.7%)
Delta Optical Density (3.91 - 0 ng/mL) = 0.034 - 0.008 =	0.026
2 SD's of 0 ng/mL Standard =	0.004

Sensitivity = $\frac{0.004}{0.026}$ x 3.91 ng/mL = **0.60 ng/mL**

Linearity

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

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

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of mouse IgM 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 IgM 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 IgM determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	m IgM (ng/nL)	Intra-assay % CV	Inter-assay % CV
Low	13.9	6.7	
Medium	59.8	3.1	
High	218.9	2.0	
Low	11.3		8.5
Medium	58.2		5.0
High	201		5.6

Cross Reactivities

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 IgG_1	<0.2%
mouse IgG_{2a}	<0.1%
mouse IgG_{2b}	<0.1%
mouse IgG_3	<0.1%

Sample Recoveries

Please refer to pages 4 and 5 for Sample Handling recommendations and Standard preparation.

Mouse IgM concentrations were measured in mouse serum, Tissue Culture Media and mouse ascites fluid. Mouse IgM was spiked into the undiluted samples of these matrices which were then diluted with the appropriate diluent and assayed in the kit. The following results were obtained:

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

* See Sample Handling instructions on page 4 for details.

References

1. T. Chard, "An Introduction to Radioimmunoassay and Related Techniques, 4th Ed.", (1990) Amsterdam: Elsevier.
2. P. Tijssen, "Practice & Theory of Enyme Immunoassays", (1985) Amsterdam: Elsevier.
3. B. Alberts, et al., "Molecular Biology of the Cell", (2002) New York, NY: Garland Science Publishing.
4. E. Ashwood and C. Burtis, "Tietz Textbook of Clinical Chemistry, 3rd Edition", (1999) W.B. Saunders Company.
5. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

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