



Mouse IgM Immunoglobulin Assay

Immunoenzymetric Assay for the Measurement of Mouse IgM Catalog # F090

Intended Use

This kit is intended for use in quantitating mouse IgM. The antibodies used in this kit are specific for Mouse IgM and will not cross react significantly with most immunoglobulins from other species or with other mouse immunoglobulins. The kit is for Research and Manufacturing use only and is not intended for diagnostic use in humans or animals.

Summary and Explanation of the Test

Monoclonal antibodies are used in many applications such as diagnostic or therapeutic agents as well as affinity supports for immunoaffinity purification of various products. These applications frequently require a sensitive assay to accurately quantitate the concentration of these antibodies in complex sample matrices. The antibodies used in this kit are mouse IgM specific. They do not cross react significantly with immunoglobulins from other species or with the other mouse immunoglobulins: IgG1, IgG2a, IgG2b IgG3, or IgA. *Cygnus Technologies* provides kits for IgG1, IgG2a, IgG2b IgG3, and total mouse immunoglobulin. See ordering information on the last page.

Principle of the Procedure

The Mouse IgM assay is a two-site immunoenzymetric assay. Samples containing mouse IgM are reacted in microtiter strips coated with an affinity purified anti-IgM capture antibody. A second horse radish peroxidase (HRP) enzyme labeled goat anti-mouse IgM antibody is reacted forming a sandwich complex of solid phase antibody- mouse immunoglobulin-enzyme labeled antibody. The microtiter strips are washed to remove any unbound reactants. The substrate tetramethyl benzidine (TMB) is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and will be directly proportional to the concentration of mouse IgM present. Accurate quantitation is achieved by comparing the signal of unknowns to IgM standards assayed at the same time.

Reagents & Materials Provided

<u>Component</u>	<u>Product #</u>
Anti-Mouse IgM HRP labeled	F091
Affinity purified goat antibody conjugated to HRP in a protein matrix with preservative, 1x12mL	
Anti-Mouse IgM coated microtiter strips	F092
12x8 well strips in a bag with desiccant	
Mouse IgM Standards	F093
Mouse IgM in a bovine protein matrix with preservative. Standards at 0, 1, 4, 12, 35 and 100 ng/mL. 1mL/vial	
TMB Substrate	F005
3,3',5,5' Tetramethyl benzidine, 1x12mL	
Stop Solution	F006
0.5N Sulfuric Acid, 1x12mL	
Wash Concentrate (20X)	F004
Tris buffered saline with preservative, 1x50mL	

Storage & Stability

- * All reagents should be stored a 2°C to 8°C for stability until the expiration date printed.
- * The substrate reagent should not be used if its absorbance at 450nm is greater than 0.1.
- * Reconstituted wash solution is stable until the expiration date of the kit.

Materials & Equipment Required But Not Provided

- Microtiter plate reader spectrophotometer with dual wavelength capability at 450 & 650nm. *(If your plate reader does not provide dual wavelength analysis you may read at just the 450nm wavelength.)*
- Pipettors - 25µL and 100µL
- Repeating or multichannel pipettor - 100µL
- Microtiter plate rotator (150 - 200 rpm)
- Sample Diluent, (recommend *Cygnus* Cat. # I028)
- Distilled water
- 1 liter wash bottle for diluted wash solution

Precautions

For research or manufacturing use only. Stop reagent is 0.5N H₂SO₄. Avoid contact with eyes and skin. Flush exposed areas immediately with distilled water.

This kit should only be used by qualified personnel.

Preparation of Reagents

- * Bring all reagents to room temperature.
- * Dilute wash concentrate to 1 liter in distilled water, and label with kit lot and expiration date and store at 4°C.

Procedural Notes

1. Complete washing of the plates to remove excess unreacted reagents is essential to good assay reproducibility and sensitivity. We advise against the use of automated or other manual operated vacuum aspiration devices for washing plates as these may result in lower specific absorbances, higher non-specific absorbance, and more variable precision. The manual wash procedure described below generally provides lower backgrounds, higher specific absorbance, and better precision. If duplicate CVs are poor or if the absorbance of the "0" standard minus a substrate blank is greater than 0.2, evaluate plate washing procedure for proper performance.

2. High Dose Hook Effect may be observed in samples with very high concentrations of mouse immunoglobulin. Samples greater than 5µg/mL may give absorbances less than the 100ng/mL standard. Hook effect is indicated when absorbance of the undiluted sample is less than the diluted samples. If hook effect is possible, samples should also be assayed diluted.

3. When dilution of samples is required dilution should be performed in a diluent validated to yield acceptable background and not contaminated with mIgM. The diluent should also give acceptable recovery when spiked with known quantities of mIgM. The preferred diluent is our Cat# I-028 available in 100mL, 500mL, or 1 liter bottles. This is the same material used to prepare the kit standards. As the sample is diluted in I-028 its matrix begins to approach that of the standards thus reducing any inaccuracies caused by dilutional artifacts. Other prospective diluents must be tested for recovery by using them to dilute the 100ng/mL standard, as described in the following "Limitations" section.

Limitations

- * Certain sample matrices may interfere in this assay. Although the assay is designed to minimize matrix

interference, materials such as detergents in high concentration, high salt concentration, extremes of pH (<6.0 and >8.5), very high buffer molarity, or very high protein concentrations may give erroneous results. **For these reasons we recommend that you first establish acceptable recovery in your sample matrices by performing a dilution/recovery experiment.** This test can be very simply performed by diluting 1 part of the 100ng/mL standard supplied with the kit into 4 parts of your sample matrix that does not contain any or very low levels of mIgM. This diluted standard when assayed as an unknown should give a recovery value after correcting for any endogenous mIgM of 15 to 25 ng/mL. Consult *Cygnus Technologies* Technical Service Department for advice on how to quantitate the assay in problematic matrices.

Assay Protocol

*The assay is very robust such that assay variables like incubation times, sample size, and other sequential incubation schemes can be altered to manipulate assay performance for more sensitivity, increased upper analytical range, or reduced sample matrix interference. Before modifying the protocol from what is recommended, users are advised to contact our technical services for input on the best way to achieve your desired goals.

* The protocol specifies the use of an approved microtiter plate shaker or rotator for the immunological step. If you do not have such a device it is possible to incubate the plate without shaking however it will be necessary to extend the first immunological incubation step by about 30 minutes to achieve comparable results to the 1 hour shaking protocol. **Do not shake during the 30 minute substrate incubation step as this may result in higher backgrounds and worse precision.**

* Avoid the assay of samples containing Sodium Azide, (NaN₃) which will destroy the HRP activity of the conjugate and could result in the under-estimation of Mouse IgM levels in that sample.

* Bring all reagents to room temperature.

* Set-up plate spectrophotometer to read dual wavelength at 450nm for the test wavelength and 650nm for the reference wavelength. Blank the instrument using the zero standard wells after assay completion. If your plate reader does not have a 650nm filter, it is acceptable to read at the 450nm only.

* All standards, controls and samples should be assayed in duplicate.

* Maintain a repetitive timing sequence from well to well for all assay steps to insure that all incubation times are the same for each well.

* Make a work list for each assay to identify the location of each standard control and sample.

* If the substrate has a distinct blue color prior to the assay it may have been contaminated. If this appears to be the case, read 100µL of substrate plus 100µL of Stop Solution against a water blank. If the absorbance is greater than 0.1 it may be necessary to obtain new substrate or the sensitivity of the assay may be compromised.

* Plates should be read within 30 min. after adding stop since color will fade over time.

Protocol

1. Pipette 25µL of standards, controls and samples into wells indicated on work list.

2. Pipette 100µL of anti-Mouse IgM:HRP (#F091).

3. Transfer to rotator and incubate at 180rpm for 1 hour at room temperature, 24°C ± 4°.

4. Aspirate, wash with at least 300µL of diluted wash solution and aspirate. Wash a total of 4 times.

5. Pipette 100µL of substrate, (#F005).

6. Incubate for 30 minutes at room temperature, 24°C ± 4°.

7. Pipette 100µL of Stop Solution.

8. Read absorbance at 450/650nm, blanking on the Zero standard.

Calculation of Results

The standards may be used to construct a standard curve with values reported in ng/mL. (See Limitations section above). This data reduction may be performed through computer methods using curve fitting routines such as point to point, spline, or 4 parameter logistic fit. **Do not use linear regression analysis to interpolate values for samples as this may lead to significant inaccuracies!** Data may also be manually reduced by plotting the absorbance values of the standard on the y-axis versus concentration on the x-axis and drawing a smooth point

to point line. Absorbances of samples are then interpolated from this standard curve.

Example Data

Well #	Contents	Abs. at 450nm	Mean Abs.	ng/mL Mouse IgM
1A	Zero Std	0.000		
1B	Zero Std	0.002	0.001	
1C	1ng/mL	0.037		
1D	1ng/mL	0.032	0.035	
1E	4ng/mL	0.114		
1F	4ng/mL	0.116	0.115	
1G	12ng/mL	0.313		
1H	12ng/mL	0.287	0.300	
2A	35ng/mL	0.748		
2B	35ng/mL	0.778	0.763	
2C	100ng/mL	1.595		
2D	100ng/mL	1.635	1.615	
2E	sample 1	0.005		
2F	sample 1	0.010	0.008	0.2
2G	sample 2	0.100		
2H	sample 2	0.105	0.103	3.6

Quality Control

-Precision on duplicate samples should yield average % coefficients of variation of less than 10% for samples greater than 1ng/mL. CVs for samples < 1ng/mL may be greater than 10%.

- For optimal performance the absorbance of the substrate when blanked against water should be < 0.1.

-It is recommended that each laboratory assay appropriate quality control samples in each run to insure that all reagents and procedures are correct.

Performance Characteristics

Cygnus Technologies has validated this assay by conventional criteria as indicated below. This validation is generic in nature and is intended to supplement but not replace certain user and product or sample specific validation that should be performed by each laboratory.

Sensitivity

The lower limit of detection (**LOD**) is defined as that concentration corresponding to a signal two standard deviations above the mean of the zero standard. LOD is <0.2 ng/mL.

The lower limit of quantitation (**LOQ**) is defined as the lowest concentration where concentration coefficients of variation (CVs) are <20%.

LOQ is ~0.3 ng/mL.

Precision

Both intra (n=20 replicates) and inter-assay (n=5 assays) precision were determined on 3 pools with low (1.50ng/mL), medium (25ng/mL), and high concentrations (75ng/mL). The % CV is the standard deviation divided by the mean and multiplied by 100.

Pool	Intra assay CV	Inter assay CV
Low	4.8%	6.2%
Medium	5.0%	6.0%
High	5.5%	8.1%

Specificity/Cross-Reactivity

Cross reactivity in two site ELISAs can manifest itself as either a false increase in mouse IgM levels (positive cross reactivity) or as a false decrease in true IgM (negative cross reactivity). Animal immunoglobulin fractions at ~2mg/mL and/or undiluted sera from the various animal species shown below were tested for positive cross reactivity by assaying the sample as an unknown. Negative cross reactivity was evaluated by spiking 25 ng/mL of mouse IgM into each of the potential cross reactants and dividing the recovered value by 25 ng/mL. The antibodies used in this kit have been affinity purified to minimize cross reactivity but it is recommended that each user test their particular sample matrix material for cross reactivity in a similar experiment.

Animal Species	% Cross Reactivity
Cat	Not detectable
Chicken	Not detectable
Cow	Not detectable
Dog	Not detectable
Goat	Not detectable
Guinea pig	Not detectable
Hamster, Syrian	Not detectable
Horse	Not detectable
Pig	Not detectable
Rabbit	Not detectable
Rat	Not detectable
Sheep	Not detectable

Other mouse immunoglobulins IgG1, IgG2a, IgG2b, IgG3, and IgA were tested at 0.1mg/mL and showed no cross reactivity.

Recovery/ Interference Studies

Various buffer matrices have been evaluated by adding known amounts of the mouse IgM preparation used to make the standards in this kit. Because this assay is designed to minimize matrix interference most of these buffers yielded acceptable recovery defined as between 80-120%. In general, extremes in pH (<5.0 and >8.5) as well as some detergents like SDS and Tween can cause under-recovery. Very high concentrations of certain proteins can also interfere in accurate detection of mouse IgM. Each user should validate that their sample matrices yield accurate recovery by performing a similar

experiment. For example, this experiment can be performed by diluting one part of the 100ng/mL standard provided with this kit into 4 parts of the sample matrix in question. Recovery should be on the order of 15 to 25 ng/mL mouse IgM. Consult *Cygnus Technologies* Technical Services if you have recovery problems in your matrix.

Hook Capacity

Increasing concentrations of mouse IgM >100 ng/mL were assayed as unknowns. The hook capacity, defined as that concentration which will give an absorbance reading less than the 100ng/mL standard was 5µg/mL.

Ordering Information/ Customer Service

To place an order or to obtain additional product information contact *Cygnus Technologies* Customer Support:

Tel: 910-454-9442 Fax: 910-454-9443

Email: cygnustec@aol.com

Web site: www.cygnustechnologies.com

4701 Southport Supply Road SE, Suite 7
Southport, NC 28461 USA

For other mouse immunoglobulin kits please specify the following catalog numbers:

IgG1	Cat. # F045
IgG2a	Cat. # F046
IgG2b	Cat. # F047
IgG3	Cat. # F200
IgGTotal	Cat. # F049