Mouse IgM ELISA Kit

Item No. 502810

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/ Size	Storage Temperature
401041	Anti-Mouse IgM ELISA Strip Plate	1 ea	4°C
401043	Anti-Mouse IgM HRP Conjugate	1 vial/750 µl	4°C
401045	Mouse IgM ELISA Standard	1 vial/200 µl	4°C
400108	Immunoassay Buffer D Concentrate (5X)	2 vials/10 ml	4°C
400074	TMB Substrate Solution	1 vial/12 ml	4°C
10011355	HRP Stop Solution	1 vial/12 ml	RT
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Mouse IgM ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

The stop solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.* safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. Materials used for **Sample Preparation** (see page 12)

INTRODUCTION

Background

Immunoglobulin M (IgM) is a member of the immunoglobulin superfamily of glycoproteins and plays a central role in the adaptive immune response and in mucosal immunology.[1,2] IgM, in monomeric or oligomeric form, is a ligand for the mouse Fc Igα and Igμ receptor (Fcα/μ-R), which mediates cellular uptake of IgM-conjugated antigens, and Fcμ-R, which is important for B cell maturation.[2-5] IgM is produced primarily by naïve B cells and is expressed in its monomeric, low-affinity form on the cell surface.[1] When activated, B cells secrete pentameric high-affinity IgM, which opsonizes antigens to target them for removal by phagocytes and to activate complement *via* the classical pathway.[1,6] IgM antibodies are produced early in infection and have been used to determine exposure to a specific pathogen.[1] Hyper-IgM syndromes are characterized by elevated levels of IgM and dysfunctions in Ig class switch recombination (CSR).[7]

About This Assay

Cayman's Mouse IgM ELISA Kit is an immunometric (*i.e.* sandwich) assay that can be used for the quantification of mouse IgM in mouse plasma, serum, and urine, mouse hybridoma supernatant, and cell culture media. The standard curve spans the range of 0-500 ng/ml, with a lower limit of detection of 1.8 ng/ml.

Principle Of This Assay

This immunometric assay is based on a double-antibody "sandwich" technique. Each well of the microwell plate supplied with the kit has been coated with a polyclonal antibody specific for mouse IgM. This antibody will bind any mouse IgM introduced into the well. A second polyclonal antibody conjugated to horseradish peroxidase (HRP), which also recognizes mouse IgM, is added to the well forming a "sandwich". The "sandwich" is immobilized on the plate and the excess reagents are washed away. The concentration of mouse IgM is determined by measuring the enzymatic activity of HRP using the chromogenic substrate TMB. After a sufficient period, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of the color is directly proportional to the amount of bound HRP conjugate, which is proportional to the concentration of mouse IgM.

$$\text{Absorbance} \propto [\text{anti-mouse IgM HRP}] \propto [\text{mouse IgM}]$$

A schematic of this process is shown in Figure 1, on page 9.

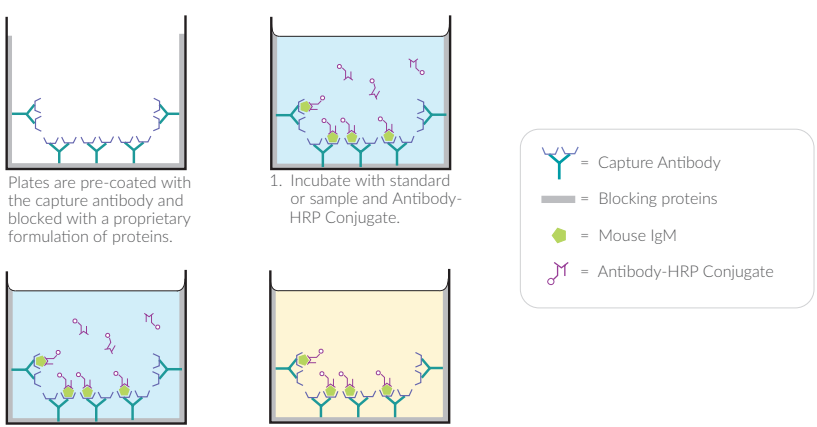


Figure 1. Schematic of the ELISA

Definition of Key Terms

Standard Curve: a plot of the absorbance values *versus* concentration of a series of wells containing various known amounts of analyte.

Lower Limit of Detection (LLOD): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

Lower Limit of Quantification (LLOQ): the lowest standard concentration in which absorbance (450 nm) – (1.64 x S.D.) is higher than the mean zero value of absorbance (450 nm) + (1.64 x S.D.).

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for at least two months. NOTE: It is normal for the concentrated buffer to contain crystalline salts. These will completely dissolve upon dilution with ultrapure water. Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

1. **Assay Buffer Preparation**

   Dilute the contents of one vial of Immunoassay Buffer D (Item No. 400108) with 40 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20 (Item No. 400035).

Sample Preparation

This assay has been validated in mouse plasma, serum, and urine, mouse hybridoma supernatant, and cell culture medium. Other sample matrices may cause interference and may require a minimal dilution. It is recommended to test for interference before embarking on a large number of measurements (see **Testing for Interference** below).

Typically, mouse plasma and serum need a minimum dilution of 500-fold to fall within the range of the standard curve. It is recommended to dilute mouse urine at least two-fold. Cell culture supernatants should be diluted with Assay Buffer to be in the range of the standard curve.

Testing for Interference

To test for interference, dilute one or two test samples to obtain several different dilutions for each sample and evaluate them in the assay. Calculate the final mouse IgM concentration for each dilution. The dilution where the final calculated mouse IgM concentration differs by 20% or less from the final concentration of the previous dilution, is the minimum required dilution for that particular sample type.

Sample Matrix Properties

Parallelism

To assess parallelism, mouse hybridoma supernatant, plasma, and serum samples were assayed at multiple dilutions using the Mouse IgM ELISA Kit. Concentrations were plotted as a function of sample dilution. The results are shown below.

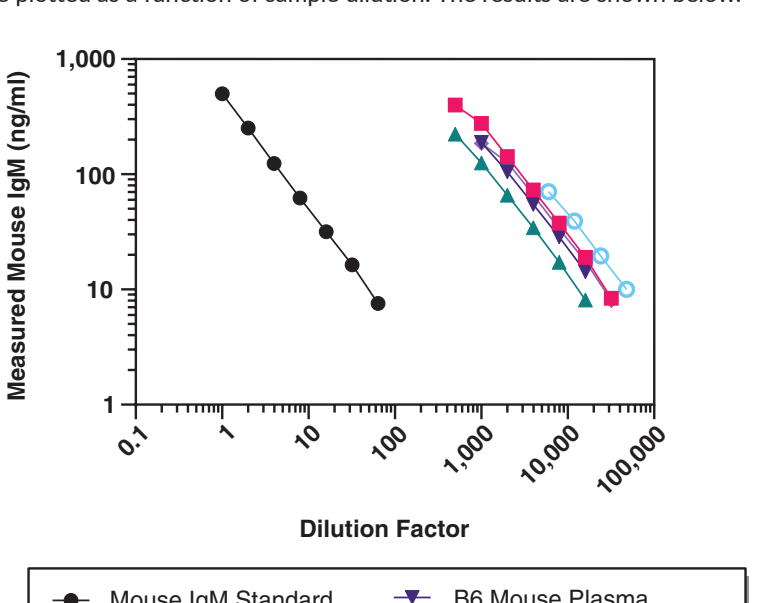


Figure 2. Parallelism of various matrices in the Mouse IgM ELISA Kit

Spike and Recovery

DMEM cell culture medium containing 10% FBS was spiked with mouse IgM, diluted with Assay Buffer, and analyzed using the Mouse IgM ELISA Kit. The results are shown below. The error bars represent the standard deviation obtained from multiple dilutions of each sample.

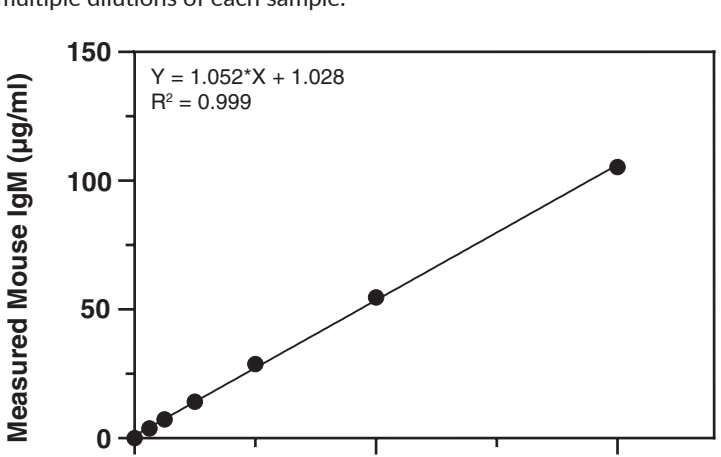


Figure 3. Spike and recovery of mouse IgM in cell culture media

Mouse urine was spiked with of mouse IgM, diluted with Assay Buffer, and analyzed using the Mouse IgM ELISA Kit. The results are shown below. The error bars represent the standard deviation obtained from multiple dilutions of each sample.

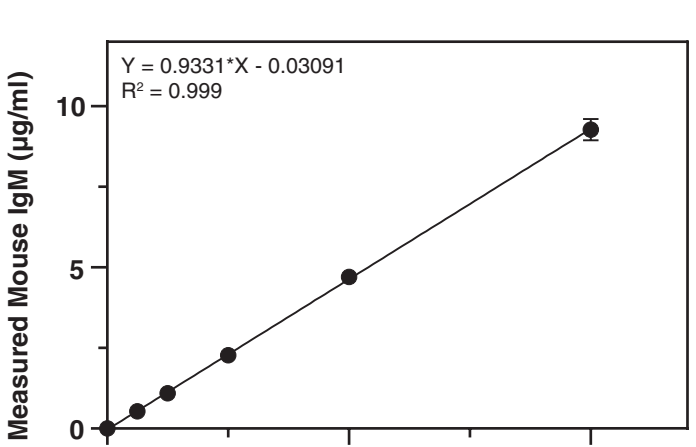


Figure 4. Spike and recovery of mouse IgM in mouse urine

Linearity

DMEM cell culture medium and mouse urine were spiked with 100 µg/ml mouse IgM and analyzed at multiple dilutions using the Mouse IgM ELISA Kit. The results are shown in the tables below.

Dilution Factor	Measured Concentration (µg/ml)	Linearity (%)
DMEM complete media		
300	105	100
600	104	99.0
1,200	104	99.0
2,400	108	103
Mouse urine		
300	84.7	100
600	80.0	94.1
1,200	78.6	92.9
2,400	81.1	95.3
4,800	84.1	98.8
9,600	82.8	97.6

Table 1. Linearity in DMEM and mouse urine
NOTE: Linearity has been calculated using the following formula:
*%Linearity = (Observed concentration value, dilution adjusted / First observed concentration value in the dilution series, dilution adjusted)*100*

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Mouse IgM Standard

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 360 µl Assay Buffer to tube #1 and 200 µl Assay Buffer to tubes #2-8. Transfer 40 µl of the Mouse IgM ELISA Standard (5 µg/ml) (Item No. 401045) to tube #1 and mix thoroughly. Serially dilute the standard by removing 200 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 200 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-7. Do not add any Mouse IgM ELISA Standard to tube #8. This tube is the background control. These diluted standards should not be stored for more than four hours.

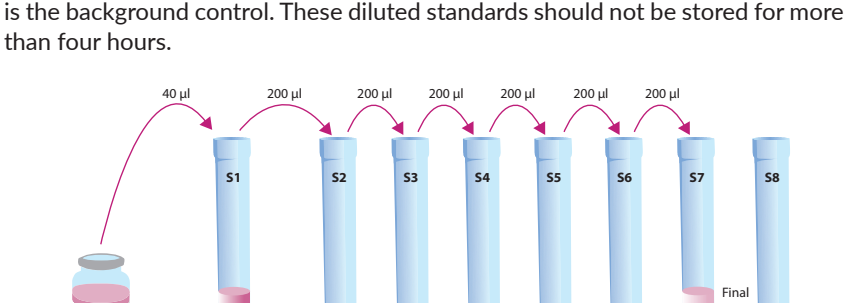


Figure 5. Preparation of the mouse IgM standards

Anti-Mouse IgM HRP Conjugate

Anti-Mouse IgM HRP Conjugate (Item No. 401043) is supplied as a concentrated (10X) stock solution of anti-mouse IgM antibody conjugated to HRP. For a full plate, add 600 µl of the HRP conjugate to 5.4 ml of Assay Buffer; for a half plate, add 300 µl of the HRP conjugate to 2.7 ml of Assay Buffer to make a 1X working solution. Do not prepare diluted HRP conjugate until immediately before use. Discard any unused HRP conjugate (1X). Store Anti-Mouse IgM HRP Conjugate (10X) stock solution at 4°C.

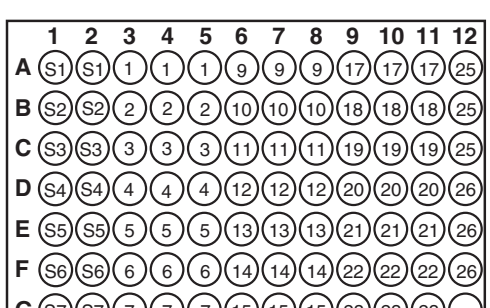
Plate Set Up

NOTE: If you do not need to use all of the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.

The 96-well plate(s) included with this kit must be pre-washed five times with Wash Buffer (1X) (~300 µl/well) prior to use in the ELISA.

Each plate or set of strips must contain an eight-point standard curve run in duplicate. Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed at least in duplicate.

A suggested plate format is shown in Figure 6, on page 19. The user may vary the location and type of wells present as necessary for each particular experiment. It is suggested that the contents of each well be recorded on the template sheet provided (see page 30).



S1-S8 = Standard Wells
1-26 = Sample Wells

Figure 6. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Pre-Wash the Plate

Rinse the plate/plate strips five times with ~300 µl/well of Wash Buffer (1X).

Addition of the Reagents

1. **Mouse IgM ELISA Standard and Samples**

   Pipette 50 µl of the Mouse IgM ELISA Standards or samples into the appropriate wells on the plate.

2. **Addition of the Anti-Mouse IgM HRP Conjugate**

   Prepare a 1X working solution of the Anti-Mouse IgM HRP Conjugate as described in the **Preparation of Assay-Specific Reagents** section. Add 50 µl of the Anti-Mouse IgM HRP Conjugate (1X) working solution to each well of the plate.

3. **Incubation of the Plate**

   Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate for one hour at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). After the last wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.
2. Add 100 µl of TMB Substrate Solution (Item No. 400074) to each well of the plate.
3. Cover the plate with the 96-Well Cover Sheet. Optimum development is obtained by using an orbital shaker at room temperature for 30 minutes, protected from light.
4. DO NOT WASH THE PLATE. Add 100 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc.*
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively, a spreadsheet program can be used.

Calculations

Plot the Standard Curve and Determine the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) *versus* concentration (linear x-axis) for standards (S1-S8) and fit the data with a quadratic fit. Using the equation of the line, calculate the concentration of mouse IgM in each sample, making sure to correct for any sample dilution.

Performance Characteristics

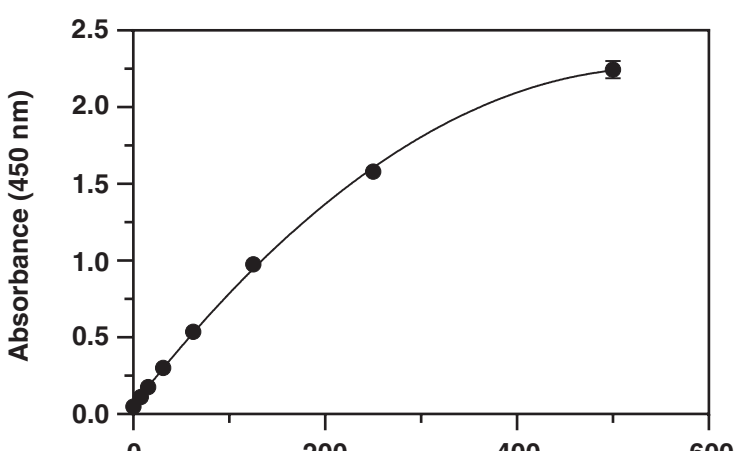
Representative Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve with each experiment. Do not use the data below to determine the values of your samples.

Mouse IgM ELISA Standards (ng/ml)	Absorbance (450 nm)	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
500	2.244	5.6	4.6
250	1.580	3.5	2.6
125	0.975	2.6	2.3
62.5	0.536	3.0	1.9
31.3	0.302	3.3	2.3
15.6	0.176	5.4	3.7
7.8	0.111	8.0	4.5
0	0.049	--	--

Table 2. Typical results

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve



Assay Range = 0-500 ng/ml
Lower Limit of Detection (LLOD) = 1.8 ng/ml
Lower Limit of Quantification (LLOQ) = 7.8 ng/ml
The standard was diluted in Assay Buffer.

Figure 7. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of three matrix controls in a single assay.

Control	Measured IgM (µg/ml)	%CV
Control 1 (BALB/c mouse plasma)	442	3.4
Control 2 (CD1 mouse plasma)	223	4.0
Control 3 (spiked cell culture medium)	4.83	2.8

Table 3. Intra-assay precision

Inter-assay precision was determined by analyzing replicates of three matrix controls in eight separate assays on different days.

Control	Measured IgM (µg/ml)	%CV
Control 1 (BALB/c mouse plasma)	291	3.0
Control 2 (CD1 mouse plasma)	158	3.4
Control 3 (spiked cell culture medium)	3.47	2.2

Table 4. Inter-assay precision

Cross Reactivity:

Compound	Cross Reactivity
Mouse IgM	100%
Mouse IgA	<0.04%
Mouse IgE	<0.04%
Mouse IgG1	<0.04%
Mouse IgG2a	<0.04%
Mouse IgG2b	<0.04%
Mouse IgG2c	<0.04%
Mouse IgG3	<0.04%

Table 5. Cross reactivity of the Mouse IgM ELISA
NOTE: No cross reactivity was detected in rabbit, goat, sheep, guinea pig, rhesus monkey, or bovine serum diluted 1:1,000 with Assay Buffer.

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of replicates	A. Trace organic contaminants in the water B. Poor pipetting/technique
High background wells (>0.150 O.D.)	A. Poor washing; ensure proper washing B. Exposure of background wells to standards or samples
Poor development (low signal) of standard curve	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents C. Conjugate has degraded D. HRP inhibitors are present
Poor development (low signal) of samples	Sample does not contain IgM antibodies or is too dilute
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present; determine minimal dilution for that sample type

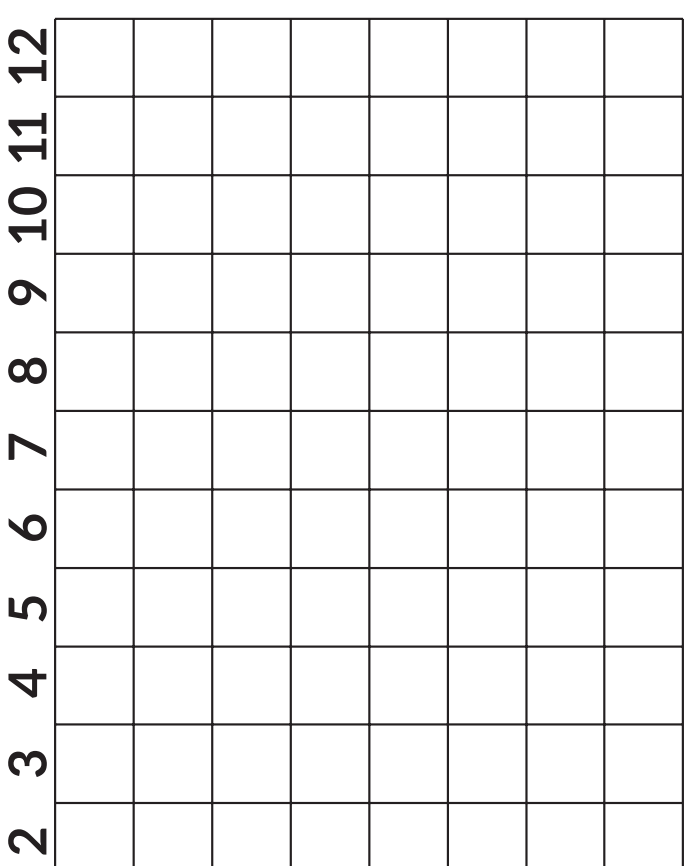
References

1. Schroeder, H.W., Jr. and Cavicini, L. Structure and function of immunoglobulins. *J. Allergy Clin. Immunol.* **125(2 Suppl. 2)**, S41-S52 (2010).
2. Li, Y., Wang, G., Li, N., *et al.* Structural insights into immunoglobulin M. *Science* **367(6481)**, 1014-1017 (2020).
3. Shibuya, A., Sakamoto, N., Shimizu, Y., *et al.* Fcα/μ receptor mediates endocytosis of IgM-coated microbes. *Nat. Immunol.*. **1(5)**, 441-446 (2000).
4. Kubagawa, H., Oka, S., Kubagawa, Y., *et al.* Identity of the elusive IgM Fc receptor (FcμR) in humans. *J. Exp. Med.* **206(12)**, 2779-2793 (2009).
5. Liu, J., Wang, Y., Min, Q., *et al.* Regulation of humoral immune responses and B cell tolerance by the IgM Fc receptor (FcμR). *Springer Nature* **1254**, 75-86 (2020).
6. Janeway, C.A., Jr., Travers, P., Walport, M., *et al.* Antibodies of different isotype operate in distinct places and have distinct effector functions. *Immunobiology: The Immune System in Health and Disease* (2001).
7. Davies, E.G. and Thrasher, A.J. Update on the hyper immunoglobulin M syndromes. *Br. J. Haematol.* **149(2)**, 167-180 (2010).

Assay Summary

Procedure	Standard/Samples
Mix all reagents gently	--
Pre-wash the plate	Wash 5 x ~300 µl/well of Wash Buffer (1X)
Add standards/samples to plate	50 µl
Add Anti-Mouse IgM HRP Conjugate (1X) working solution	50 µl
Incubate	Cover the plate and incubate for one hour at room temperature on an orbital shaker
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Add TMB Solution	100 µl
Develop	Seal plate and incubate for 30 minutes at room temperature on an orbital shaker, protected from light
DO NOT WASH. Add HRP Stop Solution	100 µl
Read	Read absorbance at 450 nm

Table 6. Assay summary



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.