

Reagents and Equipment Required:

- 1-channel pipettes covering 0-10 μ l and 200-1000 μ l
- 12-channel pipette covering 30-300 μ l
- Paper towels or kimwipes
- 50 ml tubes, 1.5 ml centrifuge tubes
- 1 N H₂SO₄
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm.

Warnings:

Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

Precautions:

- **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

Preparation of Reagents:

- **TBS:** 0.1 M Tris + 0.15 M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA in TBS
- **Wash Buffer:** The wash buffer is supplied in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.

Specimen Collection:

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 min. at 1,000 x g within 30 minutes of collection. Assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Assay Procedure:

Perform assay at room temperature. Vigorously shake plate (300 rpm) at each step of the assay.

Preparation of Standard:

Reconstitute standard by adding 1 ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1,000 ng/ml standard solution.



Dilution table for preparation of mouse IgM standard:

IgM concentration (ng/ml)	Dilutions
500	500µl (BB) + 500µl (from std vial)
250	500µl (BB) + 500µl (500ng/ml)
100	600µl (BB) + 400µl (250ng/ml)
50	500µl (BB) + 500µl (100ng/ml)
25	500µl (BB) + 500µl (50ng/ml)
10	600µl (BB) + 400µl (25ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2.5	500µl (BB) + 500µl (5ng/ml)
1	600µl (BB) + 400µl (2.5ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:

Remove microtiter plate from bag. Add 100 µl IgM standards in duplicate and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures IgM antigen in the 0.5-500 ng/ml range. If the unknown is thought to have high IgM levels, dilutions may be made in blocking buffer. A 1:5,000 to 1:10,000 dilution for normal mouse serum or plasma is suggested for best results.

Peroxidase Antibody Addition:

Reconstitute peroxidase conjugated antibody by adding 10 ml blocking buffer to vial. Agitate gently to completely dissolve contents. Add 100 µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation:

Add 100 µl TMB substrate to all wells and shake plate for 2-5 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 µl of 1 N H₂SO₄ stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly and read final absorbance values at 450 nm. For best results read plate immediately.



Measurement:

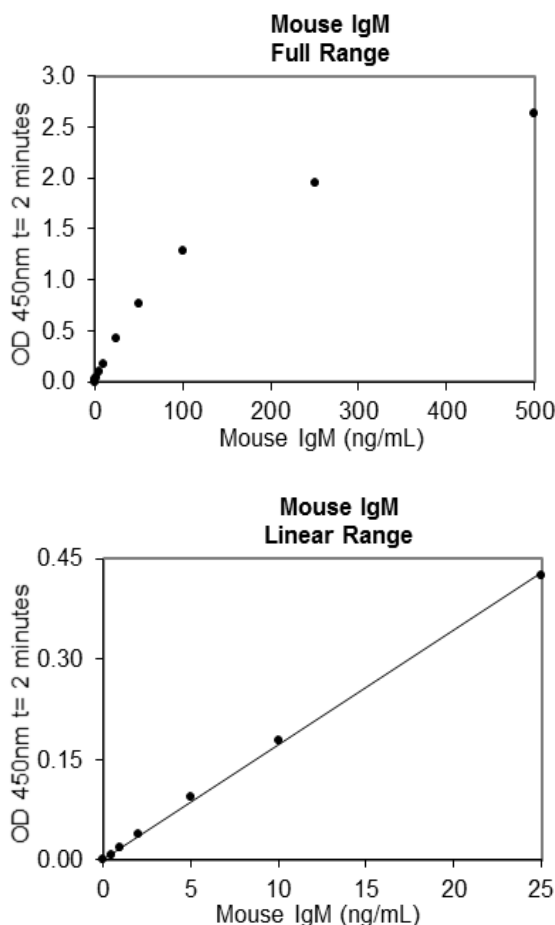
Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450 nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration:

Plot A_{450} against the amount of Mouse IgM in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of IgM in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve.

(EXAMPLE ONLY, DO NOT USE)



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Expected Values:

The concentration of IgM in normal mouse serum ranges from 0.5 to 2.0 mg/ml.

Performance Characteristics:

Sensitivity:

The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD450: 0.037-0.044) and calculating the corresponding concentration. The MDD was 0.025 ng/ml.

Sample Values:

Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean ($\mu\text{g/mL}$)
Citrate Plasma	1:2,000	335
	1:4,000	328
	1:8,000	337
	1:16,000	358



Disclaimer: This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

Example of ELISA Plate Layout

96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.5 ng/ml	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	500 ng/ml	
B	0	0.5 ng/ml	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	500 ng/ml	
C												
D												
E												
F												
G												
H												

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