

## Human IgM ELISA Kit

**Catalog No:** CS160A  
CS160B

**Size:** 1 x 96 tests  
5 x 96 tests

<b>Sensitivity:</b>	0.12 ng/ml
<b>Specificity:</b>	Total Human IgM
<b>Range:</b>	0.2-200 ng/ml
<b>Sample Type:</b>	Serum, plasma, hybridoma cell supernatants, ascites or other biological fluids
<b>Cross-Reactivity:</b>	Pooled normal plasma from Cynomolgus monkey, rabbit, pig, and horse were assayed and significant cross-reactivity was observed. Pooled normal plasma from mouse, rat, and sheep were assayed and no significant cross-reactivity was observed.

**Background:** IgM is the first immunoglobulin produced in the immune response and is the third most abundant immunoglobulin in serum. IgM is a disulfide-linked 970kDa pentamer that activates complement and is responsible for red blood cell agglutination. Each monomer consists of two mu heavy chains and two kappa or lambda light chains.

**Assay Principle:** Human IgM will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, horseradish peroxidase labeled polyclonal anti-human IgM antibody binds to the captured protein. Excess antibody is washed away and TMB substrate is used for color development at 450 nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of Human IgM. Color development is proportional to the concentration of IgM in the samples.

**Standard Calibration:** Human IgM Standard provided is calibrated against the WHO International Standard for IgM in Human Serum distributed by NIBSC (67/086), South Mimms Potters Bar, Hertfordshire, UK. Lot 616L: 1,000 ng = 0.21 IU

### Reagents Provided:

Description	Quantity
<b>CS160A – P.</b> 96-well microtiter strip plate coated with anti- Human IgM antibody, blocked and dried on well surface	1 plate: 96 wells (12 strips x 8 wells)
<b>CS160A - A.</b> Wash Buffer Concentrate (10x)	1 bottle, 50 mL
<b>CS160A - B.</b> Human IgM standard, lyophilized	1 vial
<b>CS160A - C.</b> Anti-Human IgM horseradish peroxidase antibody, lyophilized polyclonal	1 vial
<b>CS160A - D.</b> TMB substrate solution	1 bottle, 10 ml



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### Storage and Stability:

All kit components must be stored at 2-8°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 2-8°C. Kit should be used no later than the expiration date.

### Reagents and Equipment Required:

- Pipettes covering 0-10 µl and 200-1000 µl, and tips
- 12-channel pipette covering 30-300µl
- Paper towels or laboratory wipes
- Polypropylene conical 50 ml tubes, 1.5 ml flip-cap tubes
- 1N H<sub>2</sub>SO<sub>4</sub> or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)
- Deionized or distilled 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
- Automatic plate washer or wash bottle

### 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** 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 (w/v) in TBS
- **1X Wash buffer concentrate:** Dilute 50 ml of 10X wash buffer with 450 ml deionized water

### Specimen Collection:

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or aliquot and store at ≤ - 20°C. Avoid repeated freeze-thaw cycles.



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**Assay Procedure:** Allow microtiter strips and assay components to warm to room temperature for 30 minutes. Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

### Preparation of Standard:

Reconstitute Standard by adding **1.0 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.

**Table 1:** Dilution table for preparation of Human IgM standard:

IgM Concentration (ng/ml)	Dilutions
200	800 $\mu$ l (BB) + 200 $\mu$ l (from vial)
100	500 $\mu$ l (BB) + 500 $\mu$ l (200ng/ml)
50	500 $\mu$ l (BB) +500 $\mu$ l (100 ng/ml)
20	600 $\mu$ l (BB) + 400 $\mu$ l (50 ng/ml)
10	500 $\mu$ l (BB) + 500 $\mu$ l (20 ng/ml)
5	500 $\mu$ l (BB) + 500 $\mu$ l (10 ng/ml)
2	600 $\mu$ l (BB) + 400 $\mu$ l (5 ng/ml)
1	500 $\mu$ l (BB) + 500 $\mu$ l (2 ng/ml)
0.5	500 $\mu$ l (BB) + 500 $\mu$ l (1 ng/ml)
0.2	600 $\mu$ l (BB) + 400 $\mu$ l (0.5 ng/ml)
0	500 $\mu$ l (BB) Zero point to determine background

**NOTE:** Dilutions for the standard curve must be made and applied to the plate immediately.

### Standard and Unknown Addition:

Remove microtiter plate from bag. Add 100  $\mu$ l of 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  $\mu$ l wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipes.

**NOTE:** The assay measures total Human IgM antigen in the 0.2 - 200 ng/ml range. If the unknown is thought to have higher IgM levels, dilutions may be made in blocking buffer. A 1:25,000 to 1:200,000 dilution for normal Human serum or plasma is suggested for best results.

### Primary Antibody Addition:

Reconstitute antibody by adding **10 ml blocking buffer** to vial. Agitate gently to completely dissolve contents. Add 100  $\mu$ l to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300  $\mu$ l wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.



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### **Substrate Incubation:**

Add 100 µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different intensities of blue. Quench reaction by adding 50 µl of 1N H<sub>2</sub>SO<sub>4</sub> or HCl 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 by gently shaking the plate and 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<sub>450</sub>).

### **Calculation of Results:**

Plot A<sub>450</sub> against the amount of Human 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.

### **Example of ELISA Plate Layout**

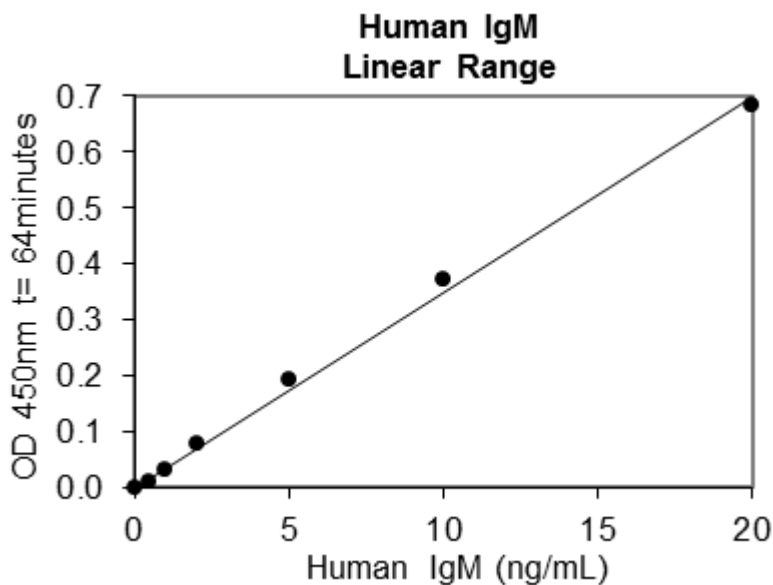
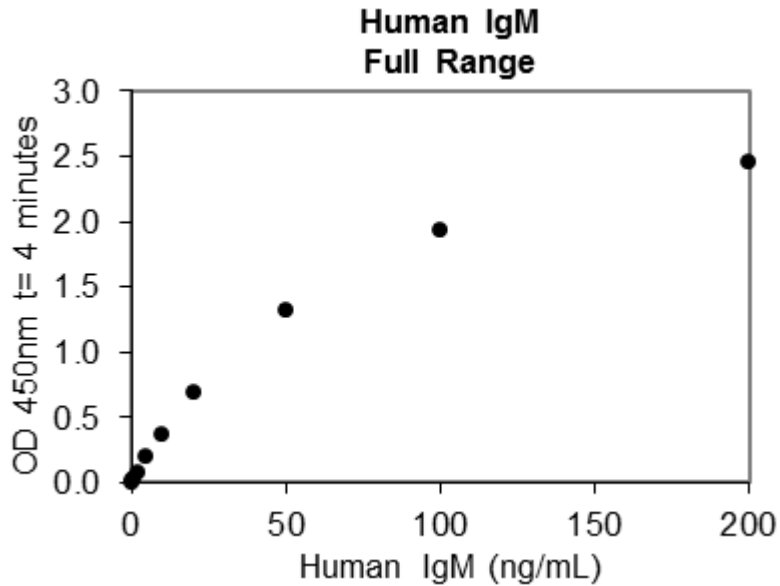
**96 Well Plate: 22 Standard wells, 74 Sample wells**

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	
B	0	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	
C												
D												
E												
F												
G												
H												



## Human IgM ELISA Kit

A typical standard curve.  
(EXAMPLE ONLY, DO NOT USE)



**Expected Value:**

The concentration of IgM in normal Human serum ranges from 0.5-2.0 mg/ml.



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**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 OD<sub>450</sub>: 0.042-0.048) and calculating the corresponding concentration. The MDD was 0.12 ng/ml.

**Specificity:** This assay recognizes total Human IgM. Pooled normal plasma from mouse, rat, and sheep was assayed and no significant cross-reactivity was observed. Pooled normal plasma from Cynomolgus monkey, rabbit, pig, and horse was assayed and significant cross-reactivity was observed.

**Intra-assay Precision:** 3 samples of known concentration were tested 20 times on 1 plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	0.778	2.09	20.2
Standard Deviation	0.057	0.102	1.29
CV (%)	7.31	4.87	6.37

**Inter-assay Precision:** These studies are currently in progress. Please contact us for more information.

**Recovery:** These studies are currently in progress. Please contact us for more information.

**Linearity:** These studies are currently in progress. Please contact us for more information.

**Sample Values:** Samples were evaluated for the presence of IgM at varying dilutions.

Sample Type	Dilution	Mean (ng/ml)
Citrate Plasma	1:25,000	802
	1:50,000	824
	1:100,000	821

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

**NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE**

