ab137982 Human IgM ELISA Kit

For the quantitative measurement of human IgM in plasma, serum, milk, urine, saliva, CSF and cell culture supernatant samples.
This product is for research use only and is not intended for diagnostic use.

Storage and Stability

Store kit at +4°C immediately upon receipt, apart from the SP Conjugate & Biotinylated Antibody, which should be stored at -20°C.

Materials Supplied

Item	Quantity	Storage Condition
IgM Microplate (12 x 8 wells)	96 wells	4°C
IgM Standard	1 vial	4°C
10X Diluent N Concentrate	30 mL	4°C
Biotinylated human IgM antibody	1 vial	-20°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	7 mL	4°C
Stop Solution	11 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Sealing Tapes	3	N/A

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 6 tubes to prepare standard or sample dilutions.

Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- If crystals have formed in the concentrate, mix gently until the crystals have dissolved.

1X Diluent N: Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1X solution gently until the crystals have completely dissolved.
Δ Note: Store for up to 1 month at 4°C.
1X Wash Buffer: Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1X solution gently until the crystals have completely dissolved.
1X Biotinylated IgM Detector antibody

1. The stock Biotinylated IgM Antibody must be diluted with 1X Diluent N according to the label concentration to prepare 1X Biotinylated IgM Antibody for use in the assay procedure. Observe the label for the "X" concentration on the vial of Biotinylated IgM Antibody.
2. Calculate the necessary amount of 1X Diluent N to dilute the Biotinylated IgM Antibody to prepare a 1X Biotinylated IgM Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation:

Number of Wells Strips	Number of Wells	(V_T) Total Volume of 1X Biotinylated Antibody (µL)
4	32	1,760
6	48	2,640
8	64	3,520
10	80	4,400
12	96	5,280

Δ Note: Any remaining solution should be frozen at -20°C.

Where:
C_S = Starting concentration (X) of stock Biotinylated IgM Antibody (variable)
C_F = Final concentration (always = 1X) of 1X Biotinylated IgM Antibody solution for the assay procedure
V_T = Total required volume of 1X Biotinylated IgM Antibody solution for the assay procedure
V_A = Total volume of (X) stock Biotinylated IgM Antibody
V_D = Total volume of 1X Diluent N required to dilute (X) stock Biotinylated IgM Antibody to prepare 1X Biotinylated IgM solution for assay procedures

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells:

$$(C_F / C_S) \times V_T = V_A$$

Calculate the final volume of 1X Diluent N required to prepare the 1X Biotinylated IgM Antibody:

$$V_T - V_A = V_D$$

Example:

Δ Note: This example is for demonstration purposes only. Please remember to check your antibody vial for the actual concentration of antibody provided.
C_S = 50X Biotinylated IgM Antibody stock
C_F = 1X Biotinylated IgM Antibody solution for use in the assay procedure
V_T = 3,520 µL (8 well strips or 64 wells)

$$(1X/50X) \times 3{,}520\ \mu L = 70.4\ \mu L$$
$$3{,}520\ \mu L - 70.4\ \mu L = 3{,}449.6\ \mu L$$

V_A = 70.4 µL total volume of (X) stock Biotinylated IgM Antibody required
V_D = 3,449.6 µL total volume of 1X Diluent N required to dilute the 50X stock Biotinylated Antibody to prepare 1X Biotinylated IgM Antibody solution for assay procedures

3. First spin the Biotinylated IgM Antibody vial to collect the contents at the bottom.
4. Add calculated amount V_A of stock Biotinylated IgM Antibody to the calculated amount V_D of 1X Diluent N. Mix gently and thoroughly.

1X SP Conjugate: Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N.
Δ Note: Any remaining solution should be frozen at -20°C.

Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the IgM Standard vial to generate a 50 ng/mL IgM Standard #1.
2. First consult the IgM Standard vial to determine the mass of protein in the vial.
3. Calculate the appropriate volume of 1X Diluent N to add when resuspending the IgM Standard vial to produce a 50 ng/mL IgM Standard #1 by using the following equation:

C_S = Starting mass of IgM Standard (see vial label) (ng)
C_F = The 50 ng/mL IgM **Standard #1** final required concentration
V_D = Required volume of 1X Diluent N for reconstitution (µL)

Calculate total required volume 1X Diluent N for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example:

Δ Note: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 165 ng of IgM Standard in vial
C_F = 50 ng/mL IgM **Standard #1** final concentration
V_D = Required volume of 1X Diluent N for reconstitution
(165 ng / 50 ng/mL) X 1,000 = 3,300 µL

4. First briefly centrifuge the IgM Standard Vial to collect the contents on the bottom of the tube.
5. Reconstitute the IgM Standard vial by adding the appropriate calculated amount VD of 1X Diluent N to the vial to generate the 50 ng/mL IgM **Standard #1.** Mix gently and thoroughly.
6. Allow the reconstituted 50 ng/mL IgM Standard #1 to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
7. Label seven tubes #2 – 8.
8. Prepare the 25 ng/mL IgM Standard #2 by adding 120 µL of the reconstituted 50 ng/mL IgM Standard #1 to 120 µL of 1X Diluent N and mix thoroughly and gently.
9. Add 120 µL of 1X Diluent N to tube #3 – 8.
10. To prepare Standard #3, add 120 µL of the Standard #2 into tube #2 and mix gently.
11. To prepare Standard #4, add 120 µL of the Standard #3 into tube #3 and mix gently.
12. Using the table below as a guide, prepare subsequent serial dilutions.

1X Diluent N serves as the zero standard (0 mg/mL).

Standard #	Volume to dilute (µL)	Volume Diluent N (µL)	Human IgM (ng/mL)
1	Step 1		50
2	120 µL Standard #1	120	25
3	120 µL Standard #2	120	12.5
4	120 µL Standard #3	120	6.25
5	120 µL Standard #4	120	3.125
6	120 µL Standard #5	120	1.563
7	120 µL Standard #6	120	0.781
8 (Blank)	N/A	120	0

Sample Preparation

Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x g for 10 minutes. Dilute samples 1:60,000 into 1X Diluent N and assay. Depending on application needs, the user should determine proper dilutions. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as an anticoagulant).

Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x g for 10 minutes and remove serum. Dilute samples 1:60,000 into 1X Diluent N and assay. Depending on application needs, the user should determine proper dilutions. The undiluted samples can be stored at -20°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute milk 1:2,000 with 1X Diluent N and assay. If necessary, dilute samples within the range of 1:1,000 to 1:20,000. Depending on application needs, the user should determine proper dilutions. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. Dilute urine 1:4 with 1X Diluent N and assay. Depending on application needs, the user should determine proper dilutions. If necessary, dilute samples within the range of 1:2 to 1:20. The undiluted samples can be stored at -20°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute 1:200 with 1X Diluent N and assay. Depending on application needs, the user should determine proper dilutions. If necessary, dilute samples within the range of 1:100 to 1:400. The undiluted samples can be stored at -20°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Cerebrospinal Fluid: Collect CSF using sample pot. Centrifuge samples at 3,000 x g for 10 minutes. Dilute samples 1:200 into 1X Diluent N or within the range of 1:2 to 1:2000, and assay. Depending on application needs, the user should determine proper dilutions. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatant: Centrifuge cell culture media at 1500 rpm for 10 minutes at +4°C to remove debris and collect supernatant. If necessary, dilute sample into MIX diluent; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13. Prepare all reagents, working standards, and samples as directed in the previous sections. The assay is performed at room temperature (20-25°C).
14. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
15. Add 50 µL of IgM Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
16. Wash five times with 200 µL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine, wash six times with 300 µL of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.
17. Add 50 µL of 1X Biotinylated IgM Antibody to each well and incubate for one hour.
18. Wash microplate as described above.
19. Add 50 µL of 1X SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
20. Wash microplate as described above.
21. Add 50 µL of Chromogen Substrate to each well. Gently tap plate thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 20 minutes or till the optimal blue color density develops.
22. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
23. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.
24. Analyze the data as described below.
    a. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
    b. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
    c. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Typical Sample Values

Sensitivity – The minimum detectable dose (MDD) of IgM as calculated by 2 standard deviations from the mean of a zero standard was established to be ~0.12 ng/ml.

Recovery

Standard Added Value: 3.125 – 25 ng/mL
Recovery: 89 – 113 %
Average Recovery: 97 %

Precision

	Intra-assay Precision	**Inter-Assay Precision**
CV (%)	4.6	8.8

Linearity of dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.
Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Dilution Factor	Plasma	Serum
1:30000	90	90
1:60000	97	102
1:120000	109	110

Assay Specificity

This kit recognizes IgM in plasma, serum, urine, saliva, milk, CSF and cell culture supernatants.
No significant cross-reactivity observed with IgA, IgA1, IgA2, IgE, IgG2, and IgJ.
10% FBS in culture media will not affect the assay.

Reference values

Normal human IgM plasma and serum levels range from 0.4 – 2.3 mg/mL.

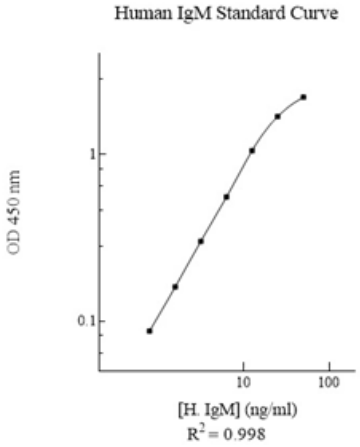
Species Reactivity

Species	% Cross Reactivity
Canine	None
Mouse	None
Monkey	2
Bovine	None
Rat	None
Swine	None
Rabbit	None
Equine	None
Protein	**Cross Reactivity (%)**
Human IgD	2
Human IgG1	1
Human IgG3	1
Human IgG4	1

- No significant cross-reactivity observed with human IgA, IgA1, IgA2, IgE, IgG2, and IGJ proteins.
- 10% FBS un culture media will not affect the assay.

Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



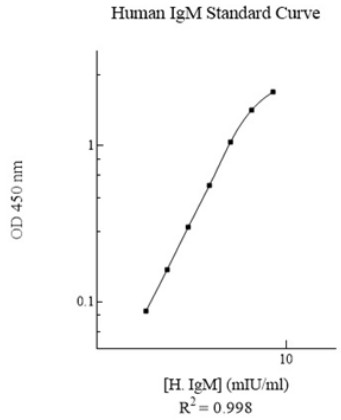


Figure 1. Example of IgM standard curve. The standard curve was prepared as described in the Standard Preparation section. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Troubleshooting

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4°C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)
High background/ Low sensitivity	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)