Human IgM ELISA Antibody Pair Kit

For detection and measurement of human immunoglobulin M

Catalog #01995 1 Kit for 6 Plates

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Product Description

The Human Immunoglobulin M (IgM) ELISA Antibody Pair Kit is intended for those who want the flexibility of setting up their own ELISA assay. The kit includes capture and detection antibodies and an IgM standard. It is designed for the quantitative detection and measurement of human IgM in biological fluids such as serum, plasma, and cell culture supernatants. IgM is predominantly found in lymph fluid and in blood where it acts as a neutralizing agent in the early stages of infection. Secreted IgM exists in a pentameric form whereas monomeric IgM is found on the surface of B cells. IgM constitutes approximately 10% of all serum immunoglobulins.

The assay is based on the sandwich ELISA method, in which samples are added to ELISA plates coated with capture antibodies specific for the immunoglobulin. The captured immunoglobulin is detected by addition of a detection antibody conjugated to alkaline phosphatase (ALP). Addition of the chromogenic enzyme substrate p-nitrophenyl phosphate (pNPP) results in a colored product with an intensity directly proportional to the concentration of immunoglobulin in the sample. The concentration of the immunoglobulin is determined by comparison to a serial dilution of the immunoglobulin standard analyzed in parallel.

NOTE: This kit includes sufficient reagents for 6 x 96-well ELISA plates. ELISA plates (Catalog #38019) and pNPP Substrate (Catalog #01917) are required for use with the Human IgM ELISA Antibody Pair Kit and are available for purchase separately.

Product Information

All components listed below are stable until expiry date (EXP) on label. Once components are opened, use within 1 month. Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated upon receipt.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	DESCRIPTION
Human IgM Standard	01995B	1 vial	Store at -20°C.	Lyophilized purified human IgM
Human IgM Capture Antibody	01995C	300 µL	Store at 2 - 8°C.	Anti-human IgM antibody (0.5 mg/mL)
Human IgM ALP-Conjugated Detection Antibody	01995D	80 µL	Store at 2 - 8°C.	ALP-conjugated anti-human IgM antibody
Standard Reconstitution Buffer B	01912	1 mL	Store at 2 - 8°C.	For reconstitution of lyophilized standard

Materials Required But Not Included

Reagents

- pNPP Substrate (Catalog #01917)
- Phosphate-buffered saline (PBS), pH 7.4 (e.g. Catalog #37350)
- PBS containing 0.05% Tween® 20 (wash buffer)
- PBS containing 0.05% Tween® 20 and 0.1% bovine serum albumin (BSA) (incubation buffer)

Equipment

- ELISA plates (e.g. Catalog #38019)
- Adhesive plate covers (e.g. Catalog #38108)
- Vertical laminar flow hood certified for Level II handling of biological materials
- Microplate reader set at a wavelength of 405 nm and (optional) a second correction wavelength of 650 nm
- ELISA plate washer: Automatic (adaptable for ELISA strip plates) or manual (e.g. multi-pipette or squirt bottle)
- Pipettor with appropriate tips
- Tubes for standard and sample dilutions; beakers, flasks, and graduated cylinders for preparation of reagents
- Timer & absorbent paper

Preparation of Reagents and Materials

A. Capture Antibody

Dilute Human IgM Capture Antibody 1 in 250 in PBS (final concentration 2 µg/mL).

Example: For one 96-well plate, add 40 µL of Human IgM Capture Antibody to 10 mL of PBS.

B. Incubation Buffer

Prepare PBS containing 0.05% Tween® 20 and 0.1% bovine serum albumin (BSA).

NOTE: For one 96-well plate, prepare 100 mL of incubation buffer.

C. Wash Buffer

Prepare PBS containing 0.05% Tween® 20.

NOTE: For one 96-well plate, prepare 750 mL of wash buffer.

D. Standard Stock Solution

1. Add 500 µL of Standard Reconstitution Buffer B to the vial of Human IgM Standard (final concentration 50 µg/mL). Let sit for 5 minutes.
2. Mix thoroughly and aliquot.

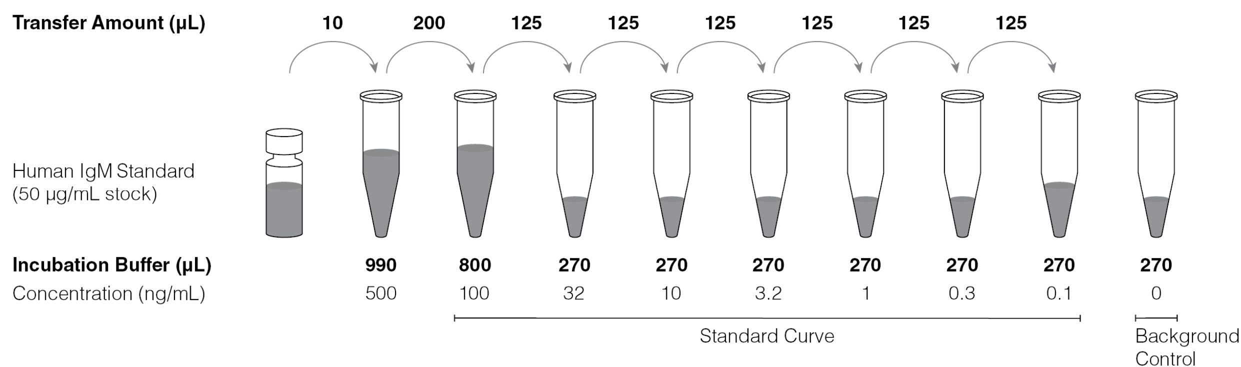
NOTE: If not used immediately, store aliquots at -20°C for up to 1 month. After thawing the aliquots, do not re-freeze.

E. Standard Curve

NOTE: The standard curve dilutions may be prepared from freshly prepared standard stock solution or from thawed aliquots. Prepare the standard curve dilutions no more than 30 minutes prior to beginning the assay. Duplicate wells for the standard curve dilutions and the background control are recommended.

Dilute the standard stock solution (prepared in section D) in incubation buffer (prepared in section B) to create standard curve dilutions ranging from 0.1 - 100 ng/mL according to Figure 1.

For the background control (0 ng/mL) use only incubation buffer.



NOTE: Volumes indicated are sufficient for duplicates.

Figure 1. Recommended Serial Dilution of Immunoglobulin Standard

F. Samples

NOTE: Avoid using lipemic, hemolysed, or contaminated samples as these may yield unreliable results.

Dilute all samples at least 1:1 in incubation buffer in tubes, for a total minimum volume of 250 µL per dilution. Samples containing high levels of immunoglobulin (exceeding the range of the standard curve) will require further dilution. Mix thoroughly.

NOTE: Duplicate wells for all samples are recommended. For samples with a high immunoglobulin concentration, it is advisable to prepare several dilutions for testing. Remove any particulate matter by centrifugation or filtration prior to use.

G. Detection Antibody

Dilute Human IgM ALP-Conjugated Detection Antibody 1 in 1000 in incubation buffer.

Example: For one 96-well plate, add 10 µL of Human IgM ALP-Conjugated Detection Antibody to 10 mL of incubation buffer.

Directions for Use

Please read the entire protocol before proceeding. Cross-contamination of reagents may invalidate assay results. Permanently labeled, dedicated, multi-channel micropipette reservoirs for reagents are recommended.

NOTE: In all washing steps, each well must be thoroughly washed and blotted dry by tapping the plate upside down on absorbent paper.

Day 1

1. Coat a high protein binding ELISA plate with Human IgM Capture Antibody (2 µg/mL; see Preparation of Reagents and Materials, section A) by adding 100 µL/well. Cover the plate with an adhesive plate cover and incubate at 2 - 8°C overnight.

Day 2

2. Bring all reagents and samples to room temperature (15 - 25°C).
3. Prepare all reagents, samples, and standard dilutions as described in Preparation of Reagents and Materials.
4. Wash the coated plate (prepared in step 1) with 2 x 300 µL of PBS/well.
5. Block the plate by adding 200 µL/well of incubation buffer.
6. Cover the plate with an adhesive plate cover and incubate at room temperature (15 - 25°C) for 1 hour.
7. Wash each well with 5 x 300 µL of wash buffer and blot dry.
8. Add 100 µL/well of each standard curve dilution and background control. Leave the blank wells empty.
9. Add 100 µL/well of diluted sample.

   NOTE: Duplicate wells for all samples are recommended.
10. Cover the plate with an adhesive plate cover and incubate at room temperature (15 - 25°C) for 2 hours.
11. Wash each well with 5 x 300 µL of wash buffer and blot dry.
12. Add 100 µL/well of diluted detection antibody. Leave blank wells empty.
13. Cover the plate with an adhesive plate cover and incubate at room temperature (15 - 25°C) for 1 hour.
14. Wash each well with 5 x 300 µL of wash buffer and blot dry.
15. Add 100 µL/well of pNPP Substrate to all wells (including blank wells).
16. Incubate at room temperature (15 - 25°C) in the dark for 60 minutes.

    NOTE: If desired, stop the reaction by adding 50 µL of 3 N NaOH solution per 200 µL of reaction mixture.
17. Measure the absorbance at 405 nm in a microplate reader. If possible, use a correction wavelength of 650 nm. Blank the reader using ELISA wells containing pNPP Substrate only.

    NOTE: Before measuring absorbance, remove any air bubbles in the wells using a small hypodermic needle or a pipette tip.
18. Calculate the average absorbance of each sample from the duplicate values. Subtract the mean absorbance value of the blank from the standard, the background control, and the sample values prior to generating the standard curve and determining the immunoglobulin concentrations of the samples. A representative standard curve is shown in Figure 2.

    NOTE: Multiply the immunoglobulin concentrations by the dilution factor used for each sample.

Performance of the Assay

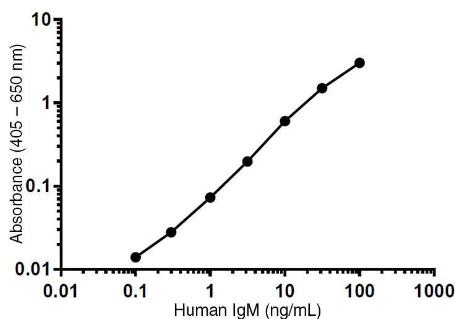


Figure 2. Representative Standard Curve

- Reportable range: 0.1 - 100 ng/mL. This is the concentration range in which measurement of the analyte can be done with the highest precision, accuracy, and linearity.
- Accuracy: The analyte standard of this ELISA was calibrated against NIBSC* international standard 67/086.

*National Institute of Biological Standards and Control, Potters Bar, Hertfordshire EN6 3QG, UK.

Troubleshooting

PROBLEM	POSSIBLE CAUSE	RECOMMENDED ACTION
Absorbance values too low	Incubation time too short	Follow the directions for the multiple incubation steps outlined in Directions for Use.
	Incorrect dilution of detection antibody	Follow the directions for dilution of detection antibody in Preparation of Reagents and Materials.
Absorbance values too high	Incubation time too long	Follow the directions for the multiple incubation steps outlined in Directions for Use.
	Temperature too high (> 25°C)	Perform assay at a lower temperature. If that is not possible, reduce incubation times.
	Incorrect dilution of detection antibody	Follow the directions for dilution of detection antibody in Preparation of Reagents and Materials.
Low absorbance readings despite good color development in wells	Incorrect wavelength setting on the ELISA reader	Check that the measuring wavelength is set at 405 nm and that the ELISA reader has the correct filter for this wavelength.
High background	Insufficient washing	Ensure that each well is washed 5X with 300 µL of wash buffer (PBS containing 0.05% Tween® 20). Ensure that multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step.
	Incorrect dilution of detection antibody	Follow the directions for dilution of detection antibody in Preparation of Reagents and Materials.
No signal with standard curve dilutions	Reagents not added in correct sequence	Follow sequence of incubation and wash steps as outlined in Directions for Use.
	Incorrect dilution of detection antibody	Follow the directions for dilution of detection antibody in Preparation of Reagents and Materials.
Inconsistent dose-response curve	Incubation conditions for individual wells not identical	Avoid delays during filling of assay wells; ensure that all reagents are at 15 - 25°C prior to beginning the assay.
Poor replicates	Insufficient washing	Ensure that each well is washed 5X with 300 µL of wash buffer (PBS containing 0.05% Tween® 20). Ensure that multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step.
	Unequal volumes in wells	Ensure that pipettes function properly.
	Carry-over between wells; evaporation from wells; splashing of well contents onto adhesive plate cover	Always use a new adhesive plate cover for each incubation. Ensure that each well is sealed tightly. Keep filled plates in a horizontal position and handle with caution.
	Samples not mixed after thawing	Vortex samples after thawing.
	High lipids or particulate matter in samples	Filter or centrifuge samples to pellet aggregates. Lipids may concentrate on the surface after centrifugation and may be removed.
	Air bubbles in well during measurement with the ELISA reader	Remove air bubbles using a small hypodermic needle or pipette tip.
Absorbance values of sample dilutions decrease with increasing concentration	Immunoglobulin concentration of the sample dilutions exceeds the upper limit of the assay	Dilute the samples further and retest along with the standard curve dilutions.

Related Products

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