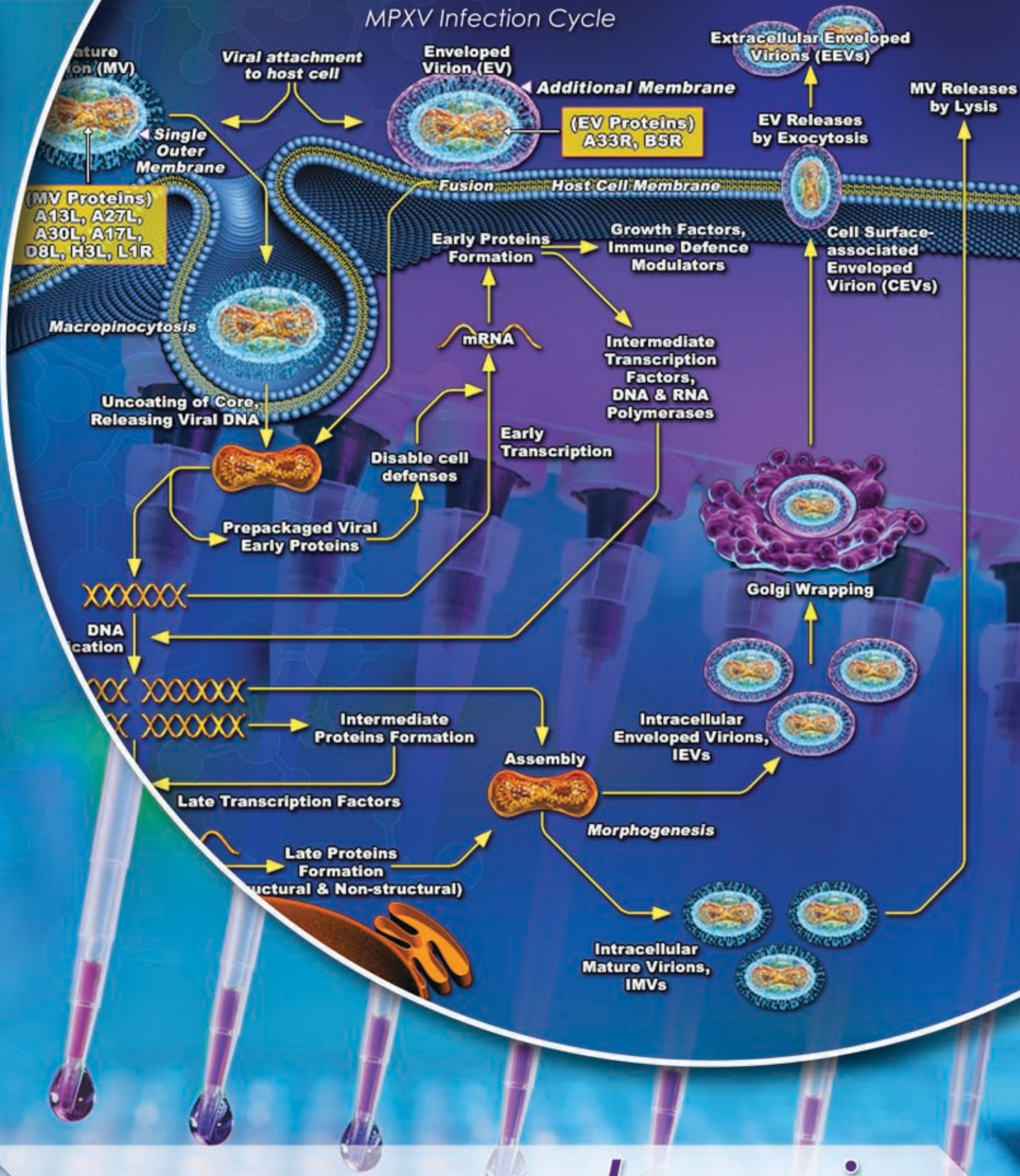
Human Anti-Monkeypox Virus IgM (Anti-MPV IgM) Pre-Coated Capture ELISA Kit



USER MANUAL

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Human Anti-Monkeypox Virus IgM (Anti-MPV IgM) Pre-Coated Capture ELISA Kit

Catalog No: 90-2395
1 × 96 well Format (96 tests)
Detection Range: 1.563 – 100 ng/ml
Sensitivity: 0.938 ng/ml

This immunoassay kit allows for the in vitro quantitative determination of Anti-MPV concentrations in 3 µl serum, 3 µl plasma and 50 µl other biological fluids.

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I. BACKGROUND

Monkeypox is a viral disease caused by monkeypox virus, a member of the orthopox virus genus. Monkeypox has two distinct branches: Branch I and Branch II. Common symptoms of monkeypox are a rash or mucosal lesions that can last for two to four weeks, along with fever, headache, muscle aches, back pain, low energy and swollen lymph nodes. Monkeypox can be transmitted to humans through physical contact with an infectious person, contaminated material, or an infected animal.

II. OVERVIEW

This kit was based on capture enzyme-linked immune-sorbent assay technology. Anti Human IgM (u chain) was pre-coated onto the 96-well plate. The HRP conjugated antigen was used as the detection antigen. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, HRP detection antigen was added to bind with Anti-MPV IgM conjugated on Anti Human IgM (u chain). After washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding acidic stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of Anti-MPV IgM in the sample is positively correlated with OD450 and can be calculated by plotting the standard curve.

III. ADVANTAGES

Multiple samples can be analyzed in a low volume, high-throughput format. Full analysis can be completed in 3 hours.

IV. STORAGE

Kit can be stored in 4°C, if you are using within a week.

If you are using within 6 months, lyophilized standard can be stored in -20°C and other components at 4°C.

V. KIT COMPONENTS

Item	Specifications	Storage
96 well Strip ELISA Plate	8 × 12 well	-20°C
Lyophilized Standard	1 vial	4°C
Sample Dilution Buffer	20 ml	4°C
HRP-labeled Antigen 100X	120 µl	4°C (in dark)
Antigen Dilution Buffer	5 ml	4°C

TMB Substrate	5 ml	4°C
Stop Solution	5 ml	4°C
25X Wash Buffer	30 ml	4°C
Plate Sealer	3 pieces	
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Material Required, (not supplied)

1. Microplate reader (wavelength: 450nm).
2. 37°C Incubator (CO2 incubator for cell culture is not recommended).
3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose).
4. Precision single (0.5-10μL, 5-50μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips (calibration is required before use).
5. Sterile tubes and Eppendorf tubes with disposable tips.
6. Absorbent paper and loading slot.
7. Deionized or distilled water.

VI. PRECAUTIONS FOR USE

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together.

VII. SAMPLE COLLECTION AND STORAGE

The following sample processing steps are concise operations. For detailed sample preparation guideline, please refer to the Quick Mark or the link.

1. **Serum:** Place whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20min at 1000xg and collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

2. **Plasma:** EDTA-Na_2/K_2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000×g 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay. For other anticoagulant types and uses, please refer to the sample preparation guideline.

3. **Tissue Sample:** Generally tissue samples are required to be made into homogenization. Protocol is as below:

   3.1. Place the target tissue on the ice. Remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Then weigh for usage.

   3.2. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue. Usually, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS (e.g. 1mM PMSF).

   3.3. Do further process using ultrasonic disruption or freeze-thaw cycles (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.) to get the homogenates.

   3.4. Homogenates are then centrifuged for 5 minutes at 5000×g. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

   3.5. Determine total protein concentration by BCA kit for further data analysis. Usually, total protein concentration for Elisa assay should be within 1-3mg/ml. Some tissue samples such as liver, kidney, pancreas which containing a higher endogenous peroxidase concentration may react with TMB substrate causing false positivity. In that case, try to use 1% H_2O_2 for 15min inactivation and perform the assay again.

Notes: PBS buffer or the mild RIPA lysis can be used as lysates. While using RIPA lysis, make the PH=7.3. Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT due to their severe inhibition for kits' working. We recommend using 50mM Tris+0.9%NaCL+0.1%SDS, PH7.3. You can prepare by yourself or contact us for purchasing.

4. **Cell Culture Supernatant:** Collect the supernatant. Centrifuge at 2500 rpm at 2-8°C for 5 minutes, then collect clarified cell culture supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

5. **Cell Lysate:**

   5.1. Suspension Cell Lysate: Centrifuge at 2500 rpm at 2-8°C for 5 minutes and collect cells. Then add pre-cooling PBS into collected cell and mix gently. Recollect cell by repeating centrifugation. Add 0.5 - 1ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Lyse the cell on ice for 30min-1h or disrupt the cell by ultrasonic disruption.

   5.2. Adherent Cell Lysate: Absorb supernatant and add pre-cooling PBS to wash three times. Add 0.5-1ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Scrape the adherent cell with cell scraper. Lyse the cell suspension added in the centrifuge tube on ice for 30min-1h or disrupt the cell by ultrasonic disruption.

   5.3. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Mucilaginous product is DNA which can be disrupted by ultrasonic cell disruptor on ice. (3~5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working).

   5.4. At the end of lysate or ultrasonic disruption, centrifuge at 10000rpm at 2-8°C for 10 minutes. Then, the supernatant is added into EP tube to detect immediately. Or you can aliquot the supernatant and store it at - 80°C for future's assay.

Notes: Read notes in tissue sample.

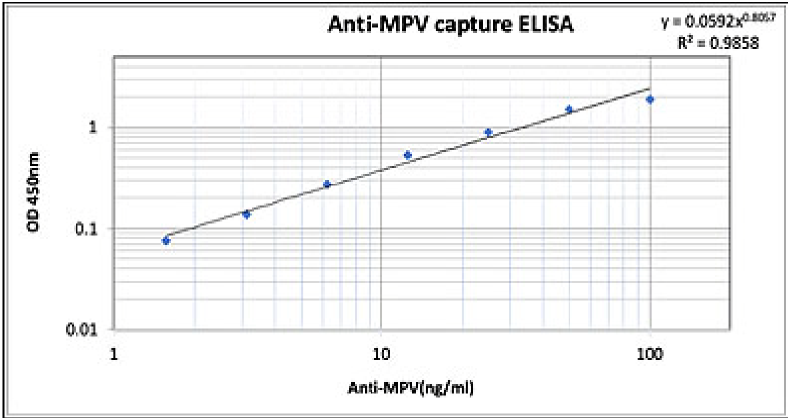
6. **Other Biological Sample:**

   Centrifuge samples for 15 minutes at 1000×g at 2-8°C. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

VIII. STANDARD CURVE

Human Anti-MPV Standard: This product has been tested by Quality Control Department and meets performance specifications mentioned in the manual. (The humidity in the laboratory is 20%-60%, and the temperature is 18°C-25°C. TMB was balanced to 37°C before color development, and incubated at 37°C for 15 minutes in the dark after adding

the enzyme label plate holes). The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.



X	ng/ml	100	50	25	12.5	6.25	3.125	1.56	0
Y	O.D.450	2.1	1.6	1.0	0.626	0.368	0.234	0.172	0.096

IX. REAGENT PREPARATION AND STORAGE

Included buffers and reagents are optimized for use with this kit. Substitution with other reagents is not recommended and may not give optimal results.

Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature (18°-25°C). For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.

1. Wash Buffer

Dilute 30ml concentrated wash buffer to 750ml wash buffer with deionized or distilled water and mix well. (The recommended resistivity of ultrapure water is 18MΩ.) Alternatively, take appropriate amount of concentrated wash buffer according to the assay requirement, then create a 25-fold dilution and mix well. Store the rest solution at 2°-8°C. Crystals formed in the concentrated wash buffer can be heated by water bath at

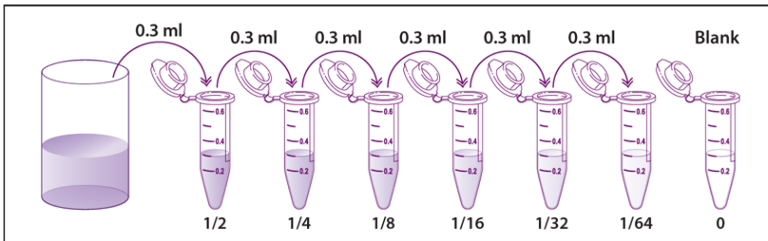
40°C till complete dissolution. (Heating temperature should be below 50°C.) Mix well for the next step. It's better to use up the prepared wash buffer in one day. Store the rest buffer at 2°-8°C within 48h.

2. **Standards**

2.1. Centrifuge the standard tube for 1min at 1000xg, making the liquid towards the bottom of tube. Label it as Zero tube.

2.2. Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the sample dilution buffer into each tube. Add 0.3ml solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 0.3ml from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 0.3ml from 1/4 tube into 1/8 tube and mix them thoroughly, so on till 1/64 tube. Now blank tube only contain 0.3ml sample dilution buffer. The standard concentration from zero tube to blank tube is 200ng/ml, 100ng/ml, 50ng/ml, 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.125ng/ml, 0ng/ml.

Figure 1: Dilution tubes



Note: Standard Solutions are best used within 2 hrs. Standard solution should be stored at 4°C for up to 12 hrs. or store at -20°C for up to 48 hrs. Avoid repeated freeze-thaw.

3. **Sample Preparation and Storage:**

Test samples should be collected, analyze immediately (within 2 hrs.) or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

3.1. Cell culture supernatants: Centrifuge to remove precipitate, analyze immediately or aliquot and store at -20°C.

3.2. Serum: Coagulate the serum at room temp about 1 hr. Centrifuge approximately 1000 x g for 15 min. Analyze serum immediately or aliquot and store at -20°C.

3.3. Plasma: Collect plasma with heparin or EDTA as the anti-coagulant. Centrifuge for 15 min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10,000 x g. Analyze immediately or aliquot and store frozen at -20°C.

3.4. Tissue Homogenates: For general information, hemolysis blood may affect the results, you should rinse the tissues with ice cold PBS (0.01M, pH 7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces. This will be homogenized in PBS in a cold glass homogenizer. (Volume depends on the weight of the tissue, 1 gram of tissue requires 9 ml of ice clod PBS with protease inhibitor). To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze- thaw cycle. Homogenates are then centrifuge for 5 min. at 5000 x g to get the supernatant.

Note: Samples to be used within 5 days may be store at 4°C, otherwise sample should be stored at -20°C (< 1 month) or -80°C (< 2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this Assay.

3.5. End user should estimate the concentration of the target protein in the test samples first, then select proper dilution factor to make the dilutaed target protein concentration falls the optimal detection range of the kit. Dilute the samples with the provided dilution buffer. Several trials may be necessary in practice. The test sample should be well mixed with the dilution buffer. Standard curve and sample should be made before the experiment.

High target protein concentration (10-100) ng/ml: Dilute 1:100 (add 1 µl of sample into 99 µl of sample/ standard dilution buffer)

Medium target protein concentration (1-10) ng/ml: Dilute 1:10 (add 10 µl of sample into 90 µl of sample/ standard dilution buffer).

Low target protein concentration (15.6-1000)pg/ml: Dilute 1:2 (add 50 µl of sample into 50 µl of sample/ standard dilution buffer).

Very low target protein concentration <15.6 pg/ml: Do not dilute, use 100 ul of sample or 1:2 dilution.

4. Preparation of HRP-labeled Antibody Working Solution:

The working solution should be prepared within 30min before the assay and can't be stored for a long time.

4.1. Calculate required total volume of the working solution: 50µl/well x quantity of wells. (It's better to prepare additional 100µl-200µl.)

4.2. Centrifuge for 1min at 1000xg in low speed and bring down the concentrated biotin-labeled antibody to the bottom of tube.

4.3. Dilute the HRP detection antibody with antibody dilution buffer at 1:99 and mix them thoroughly. (e.g. Add 10µl concentrated HRP-labeled antibody into 990µl antibody dilution buffer.)

5. **Preparation of 1 X Wash buffer:**

Prepare 1 X Wash buffer by diluting 25X Wash buffer in sterile water. Diluted Wash buffer may be stored at 4°C, however we recommend preparing fresh 1X wash buffer for each experiment.

For example: 10 ml of 25X Wash buffer in 240 ml of sterile water.

X. ASSAY PROCEDURE

When diluting samples and reagents, they must be mixed completely. It's recommended to plot a standard curve for each test.

1. Set standard, pilot samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors. Wash plate 2 times before adding standard, sample and control (blank) wells!
2. Standards and samples loading: Aliquot 50µl of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube into each standard well. Also add 50µl sample dilution buffer into the control (blank) well. Then, add 50µl pilot samples into each sample well. Seal the plate and static incubate for 30 minutes at 37°C. (Add the solution to the bottom of each well. Mix gently and without touch the sidewall and foam the sample.)
3. Wash three times: Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 350µl wash buffer into each well and immerse for 1 min. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step three times.
4. HRP-labeled Antigen: Add 50µl HRP-labeled antigen working solution into each well. Seal the plate and static incubate for 30 minutes at 37°C.

5. Wash five times: Remove the cover, and then wash the plate with wash buffer five times. Read washing method in step 3.

6. TMB Substrate: Add 50µl TMB Substrate into each well, seal the plate and static incubate at 37°C in dark within 10-20 minutes. Run the microplate reader and preheat for 15min.

Notes: Please do not use the reagent reservoirs used by HRP couplings. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells. Weaker or stronger color intensity is unacceptable. Please refer to TMB color rendering control in page 2 or QR code for detail.

7. Stop: Keep the liquid in the well after staining. Add 50µl stop solution into each well. The color will turn yellow immediately. The order for adding stop solution and TMB substrate solution is the same.

8. OD Measurement: Read the O.D. absorbance at 450nm in a microplate reader immediately. (If your microplate reader has a choice of correction wavelength, set it to 570nm or 630nm. Correct the read value to the OD450 value minus the OD570 or OD630 value. In this way, the OD value of non-chromogenic substances can be corrected and removed, thus obtaining more accurate results. If the microplate reader does not have a 570nm or 630nm wavelength, the original OD450 value can be used.)

9. Calculation of Results

9.1. Calculate the mean OD450 value (using the original OD450 value or the corrected OD450 value) of the duplicate readings for each standard, control, and sample. Then, obtain the value of calculation by subtracting the OD450 blank.

9.2. Create a four parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. (Remove the OD450 blank during plotting.) Alternatively, you can use the curve fitting software offered by the microplate reader.

9.3. Calculate the sample concentration by substituting OD450 value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

Table-1

	Standard 1	Standard 2	3	4	5	6	7	8	9	10	11	12
A	200 ng/ml	200 ng/ml										
B	100 ng/ml	100 ng/ml										
C	50 ng/ml	50 ng/ml										
D	25 ng/ml	25 ng/ml										
E	12.5 pg/ml	12.5 pg/ml										
F	6.25 pg/ml	6.25 pg/ml										
G	3.125 pg/ml	3.125 pg/ml										
H	0	0										

XI. ASSAY PROCEDURE SUMMARY

Step 1: Wash plate 2 times before adding Standard, Sample and Control (blank) wells!

Step 2: Add 50µl standard or sample into each well, seal the plate and static incubate for 90 minutes at 37°C.

Washing: Wash the plate three times and immerse for 1 min each time.

Step 3: Add 50µl HRP-labeled antigen working solution into each well, seal the plate and static incubate for 30 minutes at 37°C.

Washing: Wash the plate five times and immerse for 1 min each time.

Step 4: Add 50µl TMB substrate solution, seal the plate and static incubate for 10-20 minutes at 37°C. (Accurate TMB visualization control is required.)

Step 5: Add 50µl stop solution. Read at 450nm immediately and calculate.

XII. TROUBLE SHOOTING:

Problem	Probable Cause	Suggestion
No signal	Forgot to add all components.	Prepare check list and add the components in the correct order.
Low signal	Not enough sample per well.	Check the protein concentration. Add more sample.
High background	Washing is not sufficient.	Wash plates thoroughly.

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