Human Parainfluenza Virus 1-5 Type IgM (PIV IgM) ELISA KIT

96 Tests

Catalogue Number: E01P0004

Store all reagents at 2-8°C

Valid Period: six months

For samples:
Cell culture fluid & body fluid & tissue homogenate
Serum or blood plasma

FOR LABORATORY RESEARCH USE ONLY! NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS! PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING! THIS IS JUST A REFERENCE PROTOCOL! PLEASE USE THE MANUAL FOUND IN YOUR ELISA KIT FOR EXACT INFORMATION!

INTENDED USE

This BG PIV IGM ELISA kit is intended for laboratory research use only and not for use in diagnostic or therapeutic procedures. The stop solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of PIV IGM in the sample, this PIV IGM ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus PIV IGM concentration. The concentration of the samples is then determined by comparing the O.D. of the samples to the standard curve.

PRINCIPLE OF THE ASSAY

The coated well immuno-enzymatic assay for the quantitative measurement of PIV IGM utilizes a multi-clonal anti-PIV IGM antibody and a PIV IGM-HRP conjugate. The assay sample and buffer are incubated together with PIV IGM conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a Microplate reader. The intensity of the color is inversely proportional to the PIV IGM concentration since PIV IGM from samples and PIV IGM-HRP conjugate compete for the anti-PIV IGM antibody binding site. Since the number of sites is limited, as more sites are occupied by PIV IGM from the sample, fewer sites are left to bind PIV IGM-HRP conjugate. Standards of known PIV IGM concentrations are run concurrently with the samples being assayed and a standard curve is plotted relating the intensity of the color (Optical Density) to the concentration of PIV IGM. The PIV IGM concentration in each sample is interpolated from this standard curve.

MATERIALS

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

1	MICROTITER PLATE	96 wells	
2	ENZYME CONJUGATE	6.0 mL	1 vial
3	STANDARD.1	0 ng/mL	1 vial
4	STANDARD.2	5 ng/mL	1 vial
5	STANDARD.3	10 ng/mL	1 vial
6	STANDARD.4	25 ng/mL	1 vial
7	STANDARD.5	50 ng/mL	1 vial
8	STANDARD.6	100 ng/mL	1 vial
9	SUBSTRATE A	6.0 mL	1 vial
10	SUBSTRATE B	6.0 mL	1 vial
11	STOP SOLUTION	6.0 mL	1 vial
12	WASH SOLUTION (x100)	10 mL	1 vial
13	INSTRUCTION	1	
14.	LYSIS BUFFER SOLUTION	6.0 mL	1 vial

NOTE: The LYSIS BUFFER SOLUTION is used only when the sample is cell culture fluid & body fluid & tissue homogenate; if the sample is serum or blood plasma, then the LYSIS BUFFER SOLUTION is a superfluous reagent.

The kinds of sample:	
sample Ⅰ:	serum or blood plasma
sample Ⅱ:	cell culture fluid & body fluid & tissue homogenate

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before a centrifugation for 15minutes at approximately 1000 x g. Remove serum and perform the assay immediately or aliquot and store samples at -20 ℃ or -80℃.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2-8℃ within 30minutes of collection. Store samples at -20℃ or -80℃.Avoid repeated freeze-thaw cycles.

Cell culture fluid and other biological fluids-Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.

NOTE: Serum, plasma, and cell culture fluid samples to be used within 7 days may be stored at 2-8℃, otherwise samples must be stored at -20℃(≤2months) or -80℃(≤6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. **DO NOT USE HEAT-TREATED SAMPLES.**

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. 100 mL and 1 liter graduated cylinders.
4. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
5. Absorbent paper.
6. 37°C incubator.
7. Distilled or deionized water.
8. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log or semi-log, or log-logit as desired.
9. Tubes to prepare standard or sample dilutions.

SAMPLE PREPARATION

1. BLUEGENE (BG) is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
2. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
5. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENTS PREPARATION

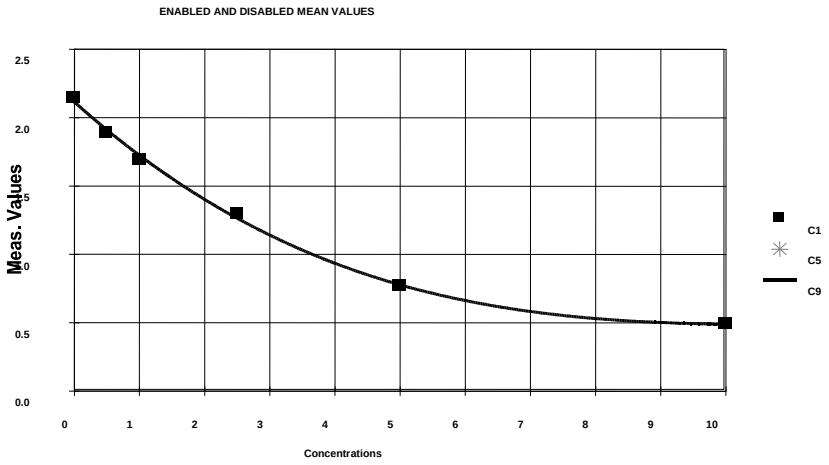
1. Bring all kit components and samples to room temperature (18-25 ℃) before use.
2. Dispense 10 μL of LYSIS BUFFER SOLUTION into 100 μL specimens, mix and stand for one hour (The proportion of LYSIS BUFFER and Specimens should be no less than 1:10). (**NOTE:** This step is required when the sample is cell culture fluid & body fluid & tissue homogenate; if the sample is serum or blood plasma, then this step should be skipped.)
3. Wash Solution - Dilute 10 mL of Wash Solution concentrate (100×) with 990 mL of deionized or distilled water to prepare 1000 mL of Wash Solution (1×).

ASSAY PROCEDURE

Prepare all Standards before starting assay procedure (**Please read Reagents Preparation)**. It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.
1. Secure the desired numbers of coated wells in the holder then add 100 μL of Standard**s** or Sample**s** to the appropriate well of the antibody pre-coated Microtiter Plate.
2. Add 50 μL of Conjugate to each well. Mix well. Mixing well in this step is important. Cover and incubate the plate for 1 hour at 37°C.
3. Wash the Microtiter Plate using one of the specified methods indicated below:
3.1 Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.
3.2 Automated Washing: Wash plate FIVE times with diluted wash solution (350-400 μL/well/wash) using an autowasher. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.
4. Add 50 μL Substrate A and 50 μL Substrate B to each well, subsequently. Cover and incubate for 10 minutes at 20-25°C. (Avoid sunlight).
5. Add 50 μL of Stop Solution to each well. Mix well.
6. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

BG CALCULATION OF RESULTS

1. This standard curve is used to determine the amount of an unknown sample. Construct a standard curve by plotting the average O.D. (450 nm) for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and draw a best fit curve through the points on the graph.
2. First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the blank control before result interpretation. Construct the standard curve using graph paper or statistical software.
3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.
4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.
5. Standard curve:



SENSITIVITY AND SPECIFICITY

1. The sensitivity in this assay is 0.01 ng/mL.
2. This assay has high sensitivity and excellent specificity for detection of PIV IGM. No significant cross-reactivity or interference between PIV IGM and analogues was observed. (Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between PIV IGM and all the analogues, therefore, cross reaction may still exist in some cases.)

SAFETY NOTES

1. This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.
2. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag and store at 2-8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. Cover or cap all reagents when not in use.
9. Do not mix or interchange different reagent lots from various kit lots.
10. Do not use reagents after the kit expiration date.
11. Read absorbances within 2 hours of assay completion.

12. The provided controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
14. Because Stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Also avoid contact between Stabilized Chromogen and metal, otherwise color may develop.
15. Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided.
16. Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well.
17. After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, and then aspirate the liquid. Repeat as directed under ASSAY PROCEDURE. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
18. Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells.
19. If using an automated washer, the operating instructions for washing equipment should be carefully followed.
20. Assay Procedure Preliminary notes: Do not mix reagents from different lots. It is recommended that assays be performed in duplicate. Standards and samples must be assayed at the same time. Avoid exposing the substrate to direct sunlight.