

# **Human Anti-Poliovirus III IgA antibody Quantitative ELISA Kit**

to determine Human Polio virus 3 IgA in Serum, Blood Plasma, Saliva,  
Urine, Tissue Liquid Samples or related Biological Solutions.

## **INSTRUCTION MANUAL**

**FOR ELISA KIT No: LTHPV3-IgA-BBA096Q**



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## **INTENDED USE**

This kit is used to determine the Human PV3 IgA in the sample of serum, blood plasma, saliva, urine, tissue liquid samples or related biological solutions.

**MANUAL VERSION 1.03**

## **INTRODUCTION**

### **BACKGROUND**

Poliovirus, the causative agent of poliomyelitis (commonly known as polio), is a human enterovirus and member of the family of Picornaviridae. Poliovirus is composed of an RNA genome and a protein capsid. The genome is a single-stranded positive-sense RN genome that is about 7500 nucleotides long. The viral particle is about 30 nm in diameter with icosahedral symmetry. Poliovirus is one of the most well-characterized viruses, and has become a useful model system for understanding the biology of RNA viruses. The three serotypes of poliovirus, PV1, PV2, and PV3, each have a slightly different capsid protein.

### **ASSAY PRINCIPLE**

Human PV3 IgA ELISA Kit quantitates Human PV3 IgA in samples. A antigen has been pre-coated onto a microplate. Standards or test samples are added to the wells, incubated and then washed. A HRP-conjugated anti-human IgA antibody is then added and incubated. The plate is washed once more and the chromogen solution is then added which HRP catalyzes, generating a blue coloration after incubation. A stop solution is added which generates conversion to yellow color read at 450 nm which is proportional to the amount of analyte bound.

### **ASSAY RESTRICTIONS**

- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.

- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

## **PRODUCT INFORMATION**

### **MATERIALS SUPPLIED**

**Human PV3 IgA microplate:** 96 well polystyrene microplates (8 strips of 12 wells) coated with the antigen.

**Human PV3 IgA standard:** Human PV3 IgA in a buffered protein base with preservatives, liquid.

**Standard diluent:** Diluent solution for reconstituted standard.

**Sample diluent:** Diluent solution for reconstituted samples.

**HRP-Conjugated anti-human IgA antibody:** liquid.

**Chromogen solution A:** liquid.

**Chromogen solution B:** liquid.

**Stop solution:** liquid.

**Wash buffer:** 30× liquid.

**Plate covers.**

### **OTHER SUPPLIES REQUIRED**

- 37 °C incubator.
- Standard microplate reader capable of measuring absorbance at 450 nm.

- Precision pipettes, disposable pipette tips and Absorbent paper.
- Distilled or deionized water.

## STORAGE CONDITIONS

Store kit reagents at 2-8 °C for 12 months. Immediately after use remaining reagents should be returned to cold storage at 4 °C. Recommended storage instructions for opened / reconstituted components are as under.

<b>KIT COMPONENTS</b>	<b>STORAGE CONDITIONS</b>
<b>Human PV3 IgA microplate</b>	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
<b>Human PV3 IgA standard</b>	Stored at 2-8 °C
<b>HRP-Conjugated anti-human IgA Antibody</b>	Stored at 2-8 °C
<b>Standard diluent</b>	Stored at 2-8 °C
<b>Sample diluent</b>	Stored at 2-8 °C
<b>Chromogen solution A</b>	Stored at 2-8 °C
<b>Chromogen solution B</b>	Stored at 2-8 °C
<b>Stop solution</b>	Stored at 2-8 °C
<b>Wash buffer</b>	Stored at 2-8 °C

## PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.

2. Allow kit reagents and materials to reach room temperature (20-25 °C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8 °C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate viruses.
10. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
11. Chromogen Solution is easily contaminated. If bluish prior to use, do not use.
12. Chromogen B contain 20% acetone, keep this reagent away from sources of heat or flame.

## **TECHNICAL HINTS**

- When mixing or reconstituting protein solutions, always avoid foaming.

- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Chromogen solution should remain colorless until added to the plate. Keep Chromogen solution protected from light. Chromogen solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Chromogen solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Chromogen solution.

## **ASSAY PROTOCOL**

### **SAMPLE COLLECTION AND STORAGE**

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Tissue homogenates** - For general information, hemolysis blood may affect the result, so 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 which will be homogenized in PBS (the volume

depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to get the supernatant.

**Cell culture supernatants and other biological fluids** - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

**Serum** - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Samples should be centrifugated adequately and no hemolysis or granule should be allowed.

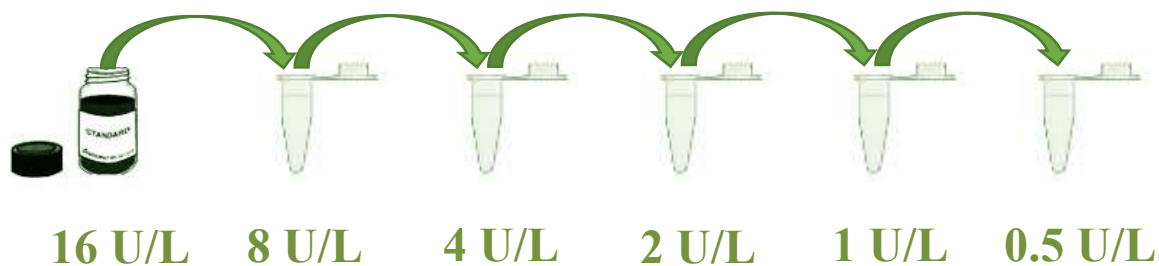
## REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in

the Buffer Concentrates, warm them gently until they completely dissolved.

**Wash buffer** - Dilute with Distilled or deionized water 1:30.

**Standard** - Pipette 150  $\mu$ L of Standard Diluent into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard. Standard Diluent serves as the zero standard.



**Note:** If samples generate values higher than the highest standard, please dilute the samples with Sample Diluent and repeat the assay.

## ASSAY PROCEDURE

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.
2. Add standard: Set Standard wells, testing sample wells. Add diluted standard 50  $\mu$ L to standard well.
3. Add Sample: Add sample diluent 40  $\mu$ L to testing sample well. Then add sample 10 ml to testing sample well; in Blank well don't add anything.
4. Cover with a plate cover and incubate for 45 minutes at 37 °C.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash buffer (250  $\mu$ L) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid

at each step is essential to good performance. After the last wash, remove any remaining Wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 50  $\mu$ L HRP-Conjugated anti-human IgA antibody to each well, except blank well.

7. Cover with a new adhesive strip. Incubate for 30 minutes at 37 °C.

8. Repeat the aspiration/wash process for five times as in step 5.

9. Add chromogen solution A 50  $\mu$ L and chromogen solution B 50  $\mu$ L to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.

10. Add 50  $\mu$ L Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

## **DATA ANALYSIS**

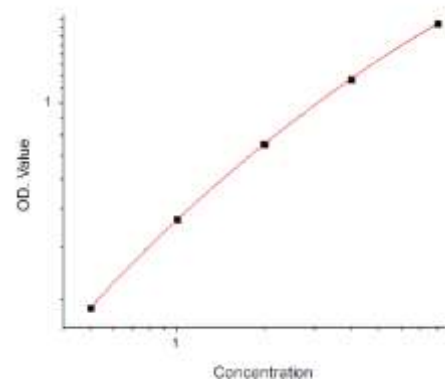
### **CALCULATION OF RESULTS**

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration for each standard on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by

plotting the log of the Human PV3 IgA concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

### **TYPICAL DATA**

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



**Detection range:** 0.5 U/L - 8 U/L

### **Intra-assay Precision** (Precision within an assay)

Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%) < 9%.

### **Inter-assay Precision** (Precision between assays)

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%) < 11%.

### **Recovery**

The recovery of Human PV3 IgA spiked to different levels in samples throughout the range of the assay in various matrices was evaluated. The

recovery ranged from 98% to 116% with an overall mean recovery of 106%.

### **Sensitivity**

The minimum detectable dose (MDD) of Human PV3 IgA is typically less than 0.05 U/L. The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

### **Linearity**

To assess linearity of the assay, samples containing and/or spiked with high concentrations of Human PV3 IgA were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

### **Specificity**

Human PV3 IgA ELISA Kit can be used to measure Human PV3 IgA in samples. Human PV3 IgA ELISA Kit has high sensitivity and excellent specificity for detection of Human PV3 IgA. No significant cross-reactivity or interference between Human PV3 IgA and analogues was observed.