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# *EDI*™ VZVAntibody (IgG) Quantitative ELISA Kit

## Enzyme Linked Immunosorbent Assay for the Quantitative Detection of Human IgG Subtype of Antibody Against Varicella-Zoster Virus

REF **KT 851** Σ **96** +2 +8 °C

**For Research Use Only. Not for Use in Clinical Diagnostic Procedures**

### INTENDED USE

This ELISA is a quantitative enzyme immunoassay for the determination of the titer of human antibody (specifically for IgG subtype) against Varicella-Zoster Virus in human specimen. Specifically, this test is useful in screening patient serum or plasma samples with extremely high titer of human anti-VZV-IgG antibody.

### SUMMARY OF PHYSIOLOGY

Varicella-Zoster Virus (VZV) is one of eight herpes viruses known to infect humans and is the etiologic agent of chicken-pox (Varicella) in children and both shingles (Zoster) and post therapeutic neuralgia in adults. Primary VZV infection results in chicken-pox, which may rarely result in complications including encephalitis or pneumonia. Even when clinical symptoms of chicken-pox have resolved, VZV remains dormant in the nervous system of the infected person, in the trigeminal and dorsal root ganglia. In about 10 – 20% of cases, VZV reactivates later in life resulting in shingles. Serious complications of shingles include postherpetic neuralgia, zoster multiplex, myelitis, herpes ophthalmicus, or zoster sine herpete. Shingles is more common in people with weakened immune systems from human immunodeficiency virus (HIV) infection, chemotherapy or radiation treatment, transplant operations and stress. In some of the above conditions, the infection of VZV may cause severe or fetal disease in patients receiving immunosuppressive therapy or may cause abnormalities in cell mediated immune response.

The presence of serum antibody to VZV has been shown to correlate with immunity to varicella. Determination of immune status to varicella is important for hospital personnel in contact with immunocompromised patients. An attenuated live VZV vaccine has been licensed in North America for individuals with non-immunocompromised disease. It is also necessary to determine the immune status of patients and evaluate their eligibility prior to administering the vaccine.

This *EDI*™ VZV-IgG ELISA kit is designed, developed and produced for the quantitative determination of relatively high titer of human anti-VZV-IgG. The antibody titer of this test are reported as kilo unit per milliliter (IU/mL).

### ASSAY PRINCIPLE

The quantitative VZV antibody (IgG) ELISA is a solid phase direct immunoassay to detect IgG antibody against VZV. Microwells are coated with VZV multiple epitope antigens. Assay standards, controls and diluted unknown serum or plasma specimens are added to the microwells. After an incubation period, the unbound antibody is washed away, and a Horseradish Peroxidase (HRP)-conjugated rabbit anti-human IgG is added to each well. The immunocomplex of well bound VZV antigen – human anti-VZV-IgG antibody – HRP-conjugated anti-human IgG will be formed. The unbound enzyme conjugates will be washed away and then the chromogen substrate solution containing hydrogen peroxide is added to all the wells. A blue color is developed with the color intensity in proportion to the amount of anti-VZV IgG antibody in the specimens. The enzyme-substrate reaction is stopped by the addition of sulfuric acid. The absorbance of assay standards, controls and unknown specimens are determined by an EIA plate reader with wavelength set at 450 nm.

### REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

**Prior to use allow all reagents to come to room temperature.** Reagents from different kit lot numbers should not be combined or interchanged.

### MATERIALS PROVIDED IN THIS KIT:

1. **VZV Antigen Coated Microplate (Cat. No. 30398)**
   One well breakable microplate with 12 x eight strips (96 wells total) coated with inactive VZV antigen. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **VZV Tracer Antibody (Cat. No. 30399)**
   One vial containing 0.6 mL concentrated horseradish peroxidase (HRP)-conjugated anti-human IgG tracer antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **VZV Tracer Antibody Diluent (Cat. No. 30400)**
   One vial containing 12 mL ready-to-use buffer. It should be only used for antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. **ELISA HRP Substrate (Cat. No. 10020)**
   One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. **ELISA Stop Solution (Cat. No. 10030)**
   One bottle contains 12 mL of 0.5 M sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

6. **Anti-VZV-IgG Calibrators (Cat. No. 30391 – 30395)**
   Five glass bottles each contain 1 mL of anti-VZV-IgG antibody in a liquid bovine serum albumin-based matrix with a non-azide preservative. **Refer to vials for exact concentration for each calibrator.** After the first use, the calibrators should be stored at

-20°C or below for long term storage. It is also allowed to be stored at 2-8°C for 2 weeks.

**7. Anti-VZV-IgG Controls (Cat. No. 30396 – 30397)**
Two glass bottles each contain 1 mL of anti-VZV-IgG antibody in a liquid bovine serum albumin-based matrix with a non azide preservative. **Refer to vials for exact concentration range for each control**. After the first use, the calibrators should be stored at -20°C or below for long term storage. It is also allowed to be stored at 2-8°C for 2 weeks.

**8. VZV Sample Diluent Concentrate, 10x (Cat. No. 30401)**
One bottle each contains 30 mL phosphate buffer with protein stabilizers and preservative. This reagent is 10-fold concentrate. It must be diluted with 270 DI-water or DT-water before use This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

**9. ELISA Wash Concentrate (Cat. No. 10010)**
One bottle contains 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.

## STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed pouch to minimize exposure to air. Use up the reagents as soon as possible after the kit is unpacked.

## SAFETY PRECAUTIONS

The reagents must be used in a research laboratory and are for research use only. The source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. Upon contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 15 µL, 50 µL, 100 µL, 200 to 1000 µL variable pipette.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm glass or plastic tubes.
5. Disposable plastic 1000 mL bottle with caps.
6. Aluminum foil.
7. Plastic microtiter well cover or polyethylene film.
8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

## SPECIMEN COLLECTION

Either serum or plasma can be used in this test. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected by venipuncture and must be allowed to clot for a minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within two hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 2 - 8ºC if the assay is to be performed within 24 hours. Otherwise, patient samples should be stored at –20ºC or below until measurement. Avoid any repeated freezing and thawing of specimen. Grossly hemolytic, lipidic or turbid samples may interfere test results and should not be used.

## ASSAY PROCEDURE

**1. Reagent Preparation**

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
(2) ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior use. Please see REAGENTS section for details.
(3) VZV Assay Buffer Concentrate (Cat. 30401) must be diluted to working solution prior use. Please see REAGENTS section for details.

**2. Assay Procedure**

(1) Place a sufficient number of VZV antigen-coated microwell strips (Cat. 30398) in a holder to run assay controls and unknown samples in duplicate.
(2) Test Configuration

| ROW | STRIP 1 | STRIP 2 | STRIP 3 |
|---|---|---|---|
| **A** | Calibrator 1 | Calibrator 5 | Unknown 2 |
| **B** | Calibrator 1 | Calibrator 5 | Unknown 2 |
| **C** | Calibrator 2 | Control A | Unknown 3 |
| **D** | Calibrator 2 | Control A | Unknown 3 |
| **E** | Calibrator 3 | Control B | |
| **F** | Calibrator 3 | Control B | |
| **G** | Calibrator 4 | Unknown 1 | |
| **H** | Calibrator 4 | Unknown 1 | |

(3) Dilute each unknown specimen in **1:3,600** before the specimen being assayed. It is suggested to do a two-step dilution for each specimen. For example, one can mix 885 µL of assay buffer with 15 µL of unknown specimen in a clean tube (D1) and further mix 885 µL of assay buffer with 15 µL of the prediluted specimen from D1 (D2). The diluted sample (D2) is ready to be measured in the following assay procedures.
(4) Add **100 µL** of assay calibrators, controls and the diluted unknown specimens into respective wells.
(5) Incubate the plate at **37°C for 30 min**.
(6) Wash each well 4 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(7) Prepare VZV Tracer Antibody Working Solution by **1:21 fold** dilution of the tracer antibody (Cat. 30399) with the Tracer Antibody Diluent (Cat. 30400). For each strip, it is required to mix 1 ml of Tracer Antibody Diluent with 50 µL of Tracer Antibody in a clean test tube.
(8) Add **100 µL** of above diluted VZV Tracer Antibody Working Solution to each well.
(9) Incubate the plate at **37°C for 30 min**.
(10) Wash each well 4 times by dispensing 350 µL of working wash solution into each well and then

completely aspirating the contents. Alternatively, an automated microplate washer can be used.

(11) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.

(12) Cover the plate with an aluminum foil to avoid exposure to light.

(13) Incubate plate at room temperature (22 – 25°C in automated system) and static for **30 minutes.**

(14) Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.

(15) Read the absorbance at **450/650 nm** within 10 minutes in a microplate reader.

## PROCEDURAL NOTES

1. It is recommended that all controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles. Store any unused antibody-coated strips in the foil Ziploc bag with desiccant to protect from moisture. Exposure of the plates to humidity drastically reduces the shelf life.
3. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
4. Incubation times or temperatures other than those stated in this insert may affect the results.
5. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
6. All reagents should be mix gently and thoroughly prior use. Avoid foaming.

## INTERPRETATION OF RESULTS

This kit contains liquid and ready to use assay calibrators with a unit of measurement per milliliter (IU/mL).

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the calibrator 1 (0 IU/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibrator curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The VZV-IgG antibody concentrations for the controls and unknown samples are read **directly from the calibration curve using their respective corrected absorbance**. If log-log graphic paper or computer assisted data reduction program utilizing logarithmic transformation are used, sample having corrected absorbance between the 1.3 IU/mL calibrator and the next highest calibrator should be calculated by the formula:

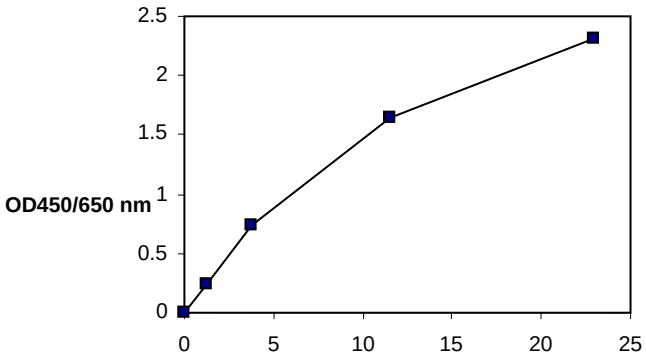
$$\text{Value of unknown} = \frac{\text{Corrected absorbance (unknown)}}{\text{Corrected Absorbance (2nd calibrator)}} \times \text{Value of the 2nd CAL}$$

## EXAMPLE DATA AND CALIBRATION CURVE

A typical absorbance data and the resulting calibration curve from this human anti-VZV-IgG antibody ELISA are represented. **This curve should not be used in lieu of standard curve run with each assay.**

| Well I.D. | Results OD 450/650 nm | |
|---|---|---|
| | Average | Corrected |
| 0 IU/mL | 0.018 | 0.000 |
| 1.3 IU/mL | 0.264 | 0.246 |
| 3.8 IU/mL | 0.751 | 0.733 |
| 11.5 IU/mL | 1.660 | 1.642 |
| 23.0 IU/mL | 2.328 | 2.310 |

**Human Anti-VZV-IgG ELISA Calibration Curve**




## LIMITATION OF THE PROCEDURE

1. This human anti-VZV-IgG antibody ELISA is limited to the quantitative measure the IgG subtype of VZV antibody in serum or plasma. As in other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. Aspirate the well or get rid of entire content of wells completely before adding the washing solution.

2. As with all diagnostic tests, a definitive diagnosis and any other decisions must not be made only on the basis of a single test. A complete evaluation by a physician is needed for a final diagnosis.

## QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known positive levels of human anti-VZV-IgG antibody. We recommend that all assays include the laboratory's own control samples in addition to those provided with this kit.

## PERFORMANCE CHARACTERISTICS

**Sensitivity**

The sensitivity of this human anti-VZV-IgG ELISA is defined as the smallest single value which can be distinguished from zero calibrator with 95% confidence level. This assay has a calculated sensitivity of 0.25 IU/mL.

**Specificity**
This ELISA is exclusively measuring the human anti-VZV-IgG antibody. There is not detectable cross-reaction to other human antibodies, such as anti-hepatitis A virus antibody, etc.

**Linearity**
Two human serum samples with relatively high level of human anti-VZV-IgG antibody were diluted with assay buffer and assayed. The results in the value of IU/mL are as follows:

| | | Measured Value (IU/mL) | Expected Value (IU/mL) | Recovery (%) |
|---|---|---|---|---|
| **Sample 1** | neat | 6.89 | - | - |
| | 1:2 | 3.12 | 3.45 | 90.4 |
| | 1:4 | 1.80 | 1.72 | 104.7 |
| | 1:8 | 0.96 | 0.86 | 111.6 |
| **Sample 2** | neat | 15.7 | - | - |
| | 1:2 | 7.00 | 7.85 | 89.2 |
| | 1:4 | 3.58 | 3.93 | 91.1 |
| | 1:8 | 1.78 | 1.96 | 90.8 |

**Recovery**
Two samples were spiked with various amounts of human anti-VZV-IgG calibrators in same volume (1 vol + 1 vol). All primary samples and were then assayed. The spike recovery results in percentage are as follows:

| Secondary spiked Sample | Primary Sample Group 1 (IU/mL) | Spiked calibrator (IU/mL) | Measured Value (IU/mL) | Expected Value (IU/mL) | Recovery (%) |
|---|---|---|---|---|---|
| **1** | 0.83 | 3 | 1.54 | 1.92 | 80.2 |
| | 0.83 | 9 | 4.94 | 4.92 | 100.4 |
| | 0.83 | 18 | 9.27 | 9.42 | 98.4 |
| **2** | 5.98 | 3 | 3.81 | 4.49 | 84.9 |
| | 5.98 | 9 | 7.21 | 7.49 | 96.3 |
| | 5.98 | 18 | 12.82 | 11.99 | 106.9 |

**Precision**
The intra-assay precision is validated by measuring two control samples in a single assay with 8-replicate determinations.

| Mean Human Anti-VZV-IgG (IU/mL) | CV (%) |
|---|---|
| 2.74 | 5.5 |
| 8.45 | 2.4 |

The inter-assay precision is validated by measuring two control samples in duplicate in 5 individual assays.

| Mean Human Anti-VZV-IgG (IU/mL) | CV (%) |
|---|---|
| 2.79 | 5.7 |
| 8.47 | 1.6 |

**High Dose "Hook" Effect**
High dose "hook" effect was check by measuring a patient sample with extremely high titer of human anti-VZV-IgG antibody in this assay. There was not any high dose "hook" effect up to sample human anti-VZV-IgG level of 1,000 IU/mL or 1,000,000 U/mL

## WARRANTY
This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

## REFERENCES

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| Catalog Number | Keep away from heat and direct sun light |
| Concentrate | Store at |
| Read instructions before use | Use by |
| | Lot No. |

## *EDI*™ Human Anti-VZV-IgG Antibody ELISA

**Short Assay Procedure:**

1. Add 100 µL of Calibrators, controls and 1:3,600 diluted specimens into the designated micro-well.
2. Incubate at 37°C for 30 min.
3. Wash each well 4 times.
4. Add 100 µL of enzyme conjugated antibody into each well.
5. Incubate at 37°C for 30 min.
6. Wash each well 4 times.
7. Add 100 µL of ELISA HRP Substrate into each of the wells.
8. Incubate at room temperature for 30 min.
9. Add 100 µL of ELISA Stop Solution into each of the wells.
10. Read the absorbance at 450nm/650 nm.

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